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IMMUNOLOGIC REACTIONS IN TISSUE CULTURES

I. THE EFFECT OF TUBERCULIN O. T. ON NORMAL LEUKOCYTES IN VITRO *

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A number of investigators have studied the effect of tubercle bacilli on the morphology of explanted tissues. Smyth [1] recorded certain changes characteristic of the early formation of tubercles in chicken-spleen and chicken-heart cultures inoculated with tubercle bacilli. According to him, clusters of leukocytes appeared about bacilli; then newly formed epithelial cells aggregated into clumps and finally fused to form giant cells. This process was followed by degeneration of the cells containing tubercle bacilli ("shadow cells"). These observations were fully corroborated and considerably extended by Maximow.[2] In order to determine the histogenesis of the epithelioid cells of the tubercle, Maximow inoculated cultures of lymphoid tissue, omentum, loose connective tissue and blood leukocytes with tubercle bacilli and studied these cultures for three weeks. He claimed an exact duplication of the tuberculous process in vitro, with tubercles, giant cells and caseation. Further corroborative evidence was brought forward by Lewis, Willis and Lewis,[3] Lang [4] and Timofejewski and Benewolenskaja.[5] These studies established a definite possibility of reproducing in vitro the typical morphologic reactions of tuberculosis.

I thought that the next step of interest would be to determine whether, coincidentally with these morphologic changes, typical immunologic reactions of tuberculosis could also be observed in explanted tissues.

In demonstrating these reactions in vitro it was decided to confine the observations as much as possible to the best known, most certain and most conspicuous reaction of this disease; namely, hypersusceptibility to tuberculin.

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* From the Laboratories of the Mount Sinai Hospital.

* This work was aided by a grant from Eugene Meyer through the Manhattan Research Foundation.

1. Smyth, H. F.: J. Exper. Med. **23**: 283, 1916.
2. Maximow, A. A.: J. Infect. Dis. **37**: 418, 1925; ibid. **34**: 549, 1924; Ann. d'anat. path. **3**: 1, 1926.
3. Lewis, M. R.; Willis, H. S., and Lewis, W. H.: Tubercle **6**: 329, 1925.
4. Lang, F. S.: J. Infect. Dis. **37**: 420, 1925.
5. Timofejewski, A. D., and Benewolenskaja, S. W.: Virchows Arch. f. path. Anat. **264**: 605, 1927.

In selecting the type of tissue to be used for the immunologic studies in vitro, preference was given to blood leukocytes on the basis of the following observations by earlier workers.

The important rôle of monocytes in the cellular reaction of the animal to tuberculosis was greatly emphasized by Sabin and her co-workers,[6] and others, and the origin of the epithelioid cells of the tubercle from these cells was established by Maximow,[2] Sabin and Doan [7] and others.

Moreover, it was recently shown by Long, Stewart and Bradley [8] that the leukocytes of tuberculous guinea-pigs underwent early death during intrapleural reactions to tuberculin. They reported that these cells themselves appeared sensitized and, therefore, seemed to participate in the phenomenon of hypersusceptibility to tuberculin.

Before attempting the studies proposed, it was necessary to determine experimentally the unknown effect of tuberculin on normal explanted leukocytes. In view of this fact, the first two papers of this series embody observations on the effect of tuberculin O.T. on cultures of the leukocytes of healthy chickens.

METHODS

Chicken leukocytes were cultured in Carrel D_8 flasks to allow prolonged observations under conditions of uninterrupted growth. The technic described by Carrel [9] was exactly followed. The white blood corpuscles were obtained by centrifugalizing, at high speed, blood immediately after its withdrawal from an adult chicken. After the removal of plasma, a few drops of diluted embryonic juice were added to the buffy coat of the sediment. In fifteen minutes, the coagulum was removed and placed in a watch glass with a small amount of Ringer's solution. After some of the red blood cells had been washed away, small fragments of coagulum containing the leukocytes were transferred to Carrel D_3 flasks. Ordinarily, two fragments were embedded in the culture medium of one flask. The culture medium consisted of 1 cc. of Tyrode solution, 0.5 cc. of chicken plasma and 0.5 cc. of diluted embryonic extract. The cultures were then incubated at 39 C. Unless otherwise stated, all the cultures were washed in Tyrode solution, patched with plasma when necessary and added with the mixture of serum, Tyrode solution and embryonic extract at regular intervals, usually after twenty-four hours of incubation and subsequently every two or three days for the rest of the time.

The tubercle bacilli employed in this work were of human and avian origin. The strain of tubercle bacilli of human type (H-37) was kindly sent to me by Dr. S. A. Petroff. The avian type was *Mycobacterium avian* strain 118 of the American Type Culture Collection. The strains were kept by means of subcultures on glycerin potato, glycerin agar and Dorsett egg medium. Moist and abundant cultures not over two months old were employed in the experiments.

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6. Cunningham, R. S.; Sabin, F. R.; Sugiayama, S., and Rindwall, S. A.: Bull. Johns Hopkins Hosp. **37**: 231, 1925; Doan, C. A., and Sabin, F. A.: J. Exper. Med. **46**: 315, 1927.
7. Sabin, F. R., and Doan, C. A.: J. Exper. Med. **46**: 627, 1927.
8. Long, F. W.; Stewart, F. W., and Bradley, J. I.: Am. J. Path. **2**: 47, 1926.
9. Carrel, A.: J. Exper. Med. **38**: 407, 1923; ibid. **36**: 365, 1922.

The tuberculin O. T. used in this work was, in part, prepared in this laboratory from strains H-37 and avian strain 118 and, in part, also obtained from the New York Board of Health. The glycerin broth cultures chosen for the preparation were six weeks old, with a thick and well wrinkled film of surface growth. Cultures with thin and smooth films were not used. Two batches of tuberculin O. T. were prepared from each glycerin broth culture: (1) the "free flame" tuberculin, in which the concentration of one tenth of the original volume was obtained by boiling the filtrate over a free flame in an open Pyrex beaker; (2) the "water bath" tuberculin, which was concentrated to one tenth of the original volume by evaporation in a water bath at 60 C.

The tuberculin of the human type was always standardized in tuberculous guinea-pigs. The guinea-pigs were infected by the subcutaneous injection of 0.1 mg. of moist weight of H-37 culture six weeks before the injection of tuberculin; 0.1 cc. of both "free flame" and "water bath" tuberculin H-37 preparations of various batches produced death in these guinea-pigs in less than twenty-four hours. The postmortem examinations showed miliary tuberculosis of the spleen, liver and lymph glands, with focal reaction at the point of primary inoculation of tubercle bacilli.

In some of the experiments B. H. tuberculin O. T. was used. This tuberculin was obtained from the New York Board of Health. According to information given by Dr. Charles Krumwiede, the tuberculin was made from strain 105 of the human type. The glycerin broth cultures were concentrated to one tenth volume by boiling in a water bath. The minimal lethal dose of this tuberculin in guinea-pigs infected with bovine tubercle bacilli from four to six weeks previously was 0.25 cc.

Dilutions of tuberculin used in the experiments were always made in Tyrode solution immediately before use, because of the possible deterioration of diluted tuberculin when it is kept for some time. Unless mentioned otherwise, 1 cc. of diluted tuberculin was added ordinarily to the surface of coagulated plasma of twenty-four hour old cultures of leukocytes. The final dilution of the tuberculin in the solid phase of the culture was not considered. The dilutions mentioned represented the actual dilution of tuberculin in Tyrode solution before addition to the culture medium. In twenty-four hours after the addition of tuberculin, the cultures were washed in Tyrode solution. The results to be described were therefore due to the contact of the diluted tuberculin with the explanted tissue for a period never longer than twenty-four hours.

Sterile glycerine broth, the same as that used for the preparation of the tuberculin, containing 5 per cent glycerine and 2 per cent peptone Witte in veal infusion broth of final p_H 7.2, was condensed to one tenth of the original volume by boiling over a free flame in an open Pyrex beaker. This broth then, properly diluted, was used as the control fluid in some of the experiments to be described.

EXPERIMENTS

In order to determine the effect of tuberculin O.T. on cultures of normal leukocytes, a number of experiments were carried out with various dilutions of different batches of tuberculin of human and avian origin and with the same dilutions of previously concentrated sterile glycerin broth.

Altogether, 226 cultures of normal leukocytes were employed for the experiments concerned with the standardization of tuberculin;

they were obtained from 30 normal chickens and several embryos; 175 were Carrel D_5 flask cultures and 51 were slide cultures.

As demonstrated by Carrel [9] the slide cultures of leukocytes were not reliable for prolonged observations, since frequent transfers were necessary and, therefore, the final outcome of each culture depended on numerous minor factors, especially on the skill with which the fragments were cut for such transfers. In view of these facts, the slide cultures were employed in this part of the work only to obtain preliminary observations and for the purpose, rather, of orientation. The various dilutions of tuberculin were added to the culture medium containing fragments of embryonic spleen, and observations made for forty-eight hours afterward. Attempts to transplant leukocytes and carry on prolonged observations were not made with such cultures.

The conditions were entirely different when Carrel's physiologic method for the cultivation of leukocytes in flasks was used. Since, under these conditions, the growth was uninterrupted, the results were reliable and consistent throughout the work. In all the experiments described in these papers, a series of cultures made from the blood of a chicken was not used for any experimental purpose until after the first twenty-four hours of cultivation. After twenty-four hours of incubation, the cultures were examined microscopically, and only those cultures which were in good condition were used for the experiments. In ascertaining the condition of a given culture, attention was paid to the degree of migration, the clearness of the protoplasm of the individual cells, the overcrowding and the spontaneous agglutination of the cells and the liquefaction of the culture medium. Since each series consisting of cultures in good condition was afterward kept under the same conditions of temperature and all of them were treated with the same batches of Tyrode solution, serum, plasma and embryonic extract, the entire series, as a rule, behaved in the same manner, and their further growths were fairly identical. Moreover, no attention was ever paid to slight variations in the condition of experimental cultures under observation, even though they belonged to the same series. In addition, at least two cultures of each series were ordinarily kept as controls. The presence of two fragments in each flask culture was also helpful, as only the results which affected both fragments to the same degree were considered consistent.

Since, as mentioned before, the leukocytic cultures were not used for experimental purposes until after twenty-four hours of cultivation, it was impossible to establish with any degree of certainty the effect of tuberculin on the polymorphonuclear neutrophil leukocytes. As demonstrated by Carrel,[9] these cells migrated for the first twenty-four to forty-eight hours, and then gradually and spontaneously disappeared. The

cellular response described in these papers concerned, therefore, only the monocytes, which multiplied and persisted, in every leukocytic culture, in the majority.

An attempt was not made to differentiate the reaction to tuberculin of desquamated endothelial cells (the presence of which in the blood was demonstrated by Sabin and Doan [10]) from that of monocytes.

From observations made under the conditions mentioned, it was soon concluded that the effect of tuberculin could be conveniently studied

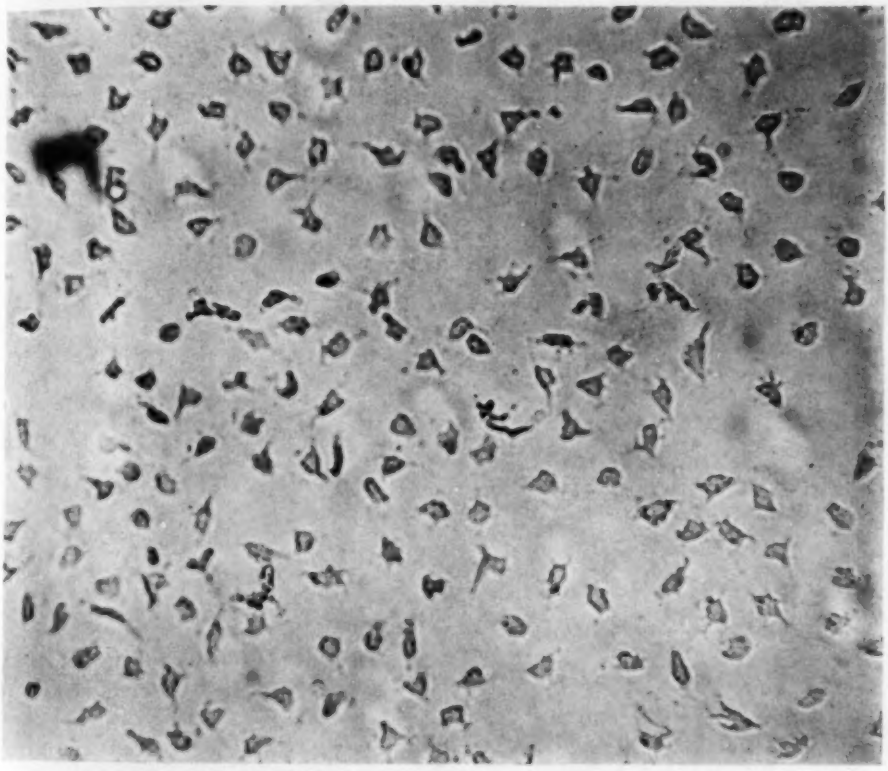

Fig. 1. Photomicrograph of unstained flask culture of normal chicken leukocytes—seventy-two hours old; × 210.

in relation to the viability of the cells and in relation to their morphologic appearance. The criteria used for determining the effect of tuberculin can be described in a general manner, as follows:

If a toxic dilution of tuberculin was added to a leukocytic culture death of the cells occurred, which expressed itself in many ways, depending on the degree of toxicity. An active living cell became round and, as a rule, considerably smaller. The protoplasm appeared dark and granular. The migration, natu-

10. Sabin, F. R., and Doan, C. A.: J. Exper. Med. **43**: 823, 1926.

rally, ceased. The dead cells located next to the original blood fragment, as well as at the peripheral part of the growth, formed large clumps. In this manner the culture appeared denser than normally. The density of the clumps depended on the quantity of disintegrated cells, and, therefore, the degree of toxicity of tuberculin could be approximately estimated from the appearance of the culture (figs. 1 and 2). In the course of the following few days an almost complete disappearance of the masses of the dead cells was frequently noticed. The dissolution of these masses was often so pronounced that only occasional surviving cells were seen in an otherwise entirely clear culture clot in the flasks. Then the surviving cells began to recover and continued to migrate and

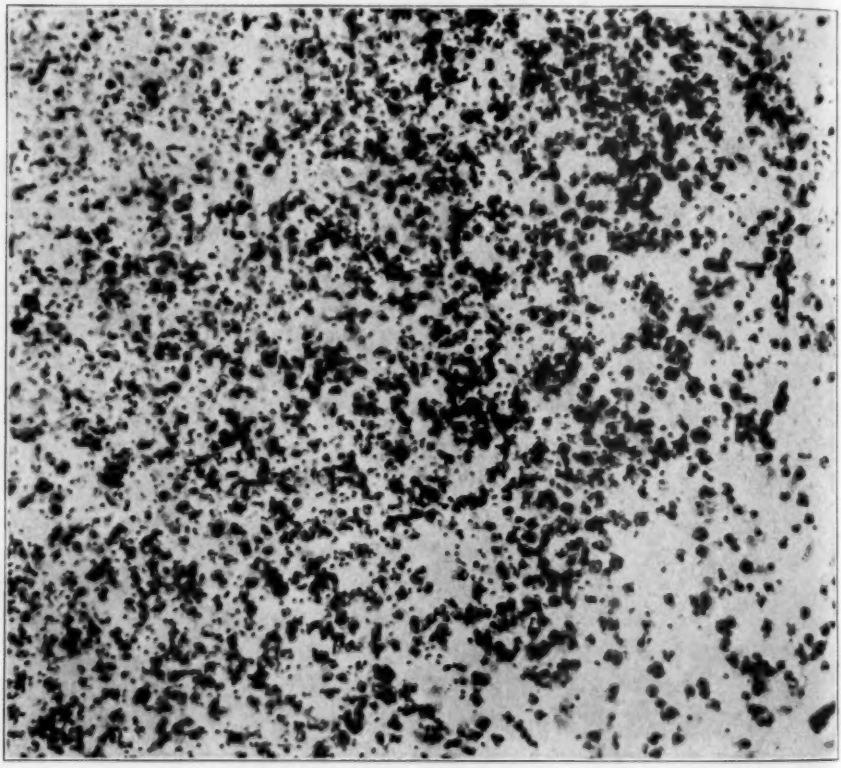

Fig. 2.—Culture of normal chicken leukocytes killed by B. H. tuberculin in a dilution of 1:300. Stained with hematoxylin; × 108.

multiply. The protoplasm of the recovered cells was of clear structure, and their activity was reestablished, as seen from the appearance of pseudopods in such cells. The new cells formed scattered small groups in various parts of the clots. The type of recovery can be briefly termed recovery with preceding dissolution of dead cells. However, the preceding dissolution of dead cells was not essential to recovery of leukocytic cultures from the toxic effect of tuberculin. In many instances, occasional surviving cells found their way through the masses of dead cells and formed a new profuse growth in another layer of the clot and among the dead cells. The clumps of dead cells gradually disappeared afterward. Thus, there was also recovery with subsequent disappearance of dead cells. A distinction was made between these two types of recovery from

the toxic effect of tuberculin, mainly because it was believed that the course depended on the character of the recovering cells. In fact, recovery of the first type was accompanied by the emergence of what appeared to be normal monocytes. They migrated slowly and remained in islands for a long time. Recovery of the second type was accompanied by emergence of hypertrophied monocytes, which were highly active. These cells invaded the clot in a short time and quickly formed continuous layers. Their morphology also appeared different. In addition, cells of still different morphology were seen in stained preparations of cultures of the second type. The morphologic changes will be discussed separately in a later part of this paper.

As is seen from this general description, the microscopic examination of unstained cultures revealed definite criteria by means of which the effect of tuberculin on the cells in vitro could be studied. These studies were pursued in a series of experiments, of which those to be described are typical.

The Influence of Glycerin Broth on Normal Leukocytes in Vitro.—It was important to determine the effect of glycerin broth on the explanted leukocytes, since this broth was used for the preparation of tuberculin.

Glycerine broth previously concentrated to one tenth of the original volume was added to a series of twenty-four hour old leukocytic cultures in dilutions of 1:200 (1) and 1:300 (2). Two cultures (3 and 4) received Tyrode solution and one culture (5) received a mixture of serum, Tyrode solution and embryonic juice. In twenty-four hours the cultures were washed in Tyrode solution and further treated in the usual manner.

Observations on this series of cultures in the course of the following two weeks definitely proved that glycerin broth in the dilutions employed did not have any toxic effect. Moreover, it seemed that the diluted glycerin broth even had a slightly stimulative effect as compared with Tyrode solution. This impression was gained from the foregoing and similar experiments. In fact, after forty-eight hours of incubation, cultures 1, 2 and 5 used in this experiment were in perfect condition. There was an area of active migration of the polymorphonuclear neutrophil leukocytes and occasional monocytes. Dead cells were not seen. Cultures 3 and 4, although in good condition, showed a smaller area of migration, and a few dead cells were noticed next to the original blood fragment. After further incubation all the cultures were in good condition and alike.

The Influence of Tuberculin O.T. on Normal Leukocytes.—After the effect of glycerin broth on normal leukocytes had been determined, attempts were made to study the effect of tuberculin which was prepared in the same, and with similar, glycerin broth. Observations were made on various batches of tuberculin O.T. prepared from strains of human and avian type.

Titration of B. H. Tuberculin in Normal Leukocytic Cultures. PROTOCOL 1.—Normal leukocytes which were in excellent condition after twenty-four hours of incubation were used. Cultures 1 and 2 received each 1 cc. of Tyrode solution. Three cultures (3, 4 and 5) received tuberculin B. H. diluted 1:300, 1:500 and 1:700. In twenty-four hours, cultures 1 and 2 were in good condition with migration of normal cells at the periphery. Cultures 3, 4 and 5 were in poor condition. Numerous dead cells were seen and there was not any migration of new cells. In forty-eight hours after the addition of tuberculin,[11] cultures

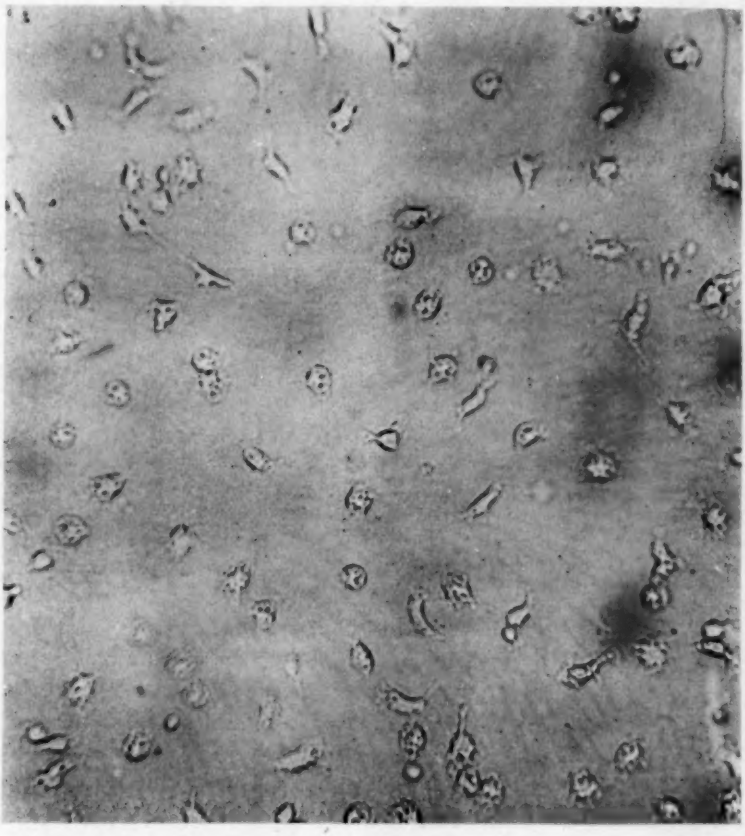

Fig. 3.—Photomicrograph of unstained flask culture of normal chicken leukocytes—seventy-two hours old. "Free flame" H-37 tuberculin (batch 2) in a dilution of 1:500 had been added to the culture at the age of twenty-four hours. The leukocytes appear granular; × 210.

1 and 2 were in good condition. Cultures 3 and 4 were dead. Culture 5 contained numerous dead cells but small groups of slightly elongated monocytes were observed, which appeared alive. During the following two days, the con-

11. It should be pointed out again that the results of this and the following experiments were due to the contact with tuberculin for a period not longer than twenty-four hours. Cultures were treated afterward in the usual manner, which was described under methods.

dition of all the cultures remained unchanged. Cultures 3 and 4, which did not show a living cell, were discarded. On the fifth day after the addition of tuberculin, culture 5 contained some exceedingly long and thin, almost threadlike monocytes among the clusters of dead cells. They appeared somewhat dark and granular. On the following day, the appearance of this culture was strikingly changed. There were fewer clumps of dead cells and an almost continuous film of the monocytes located in a layer different from that of the dead

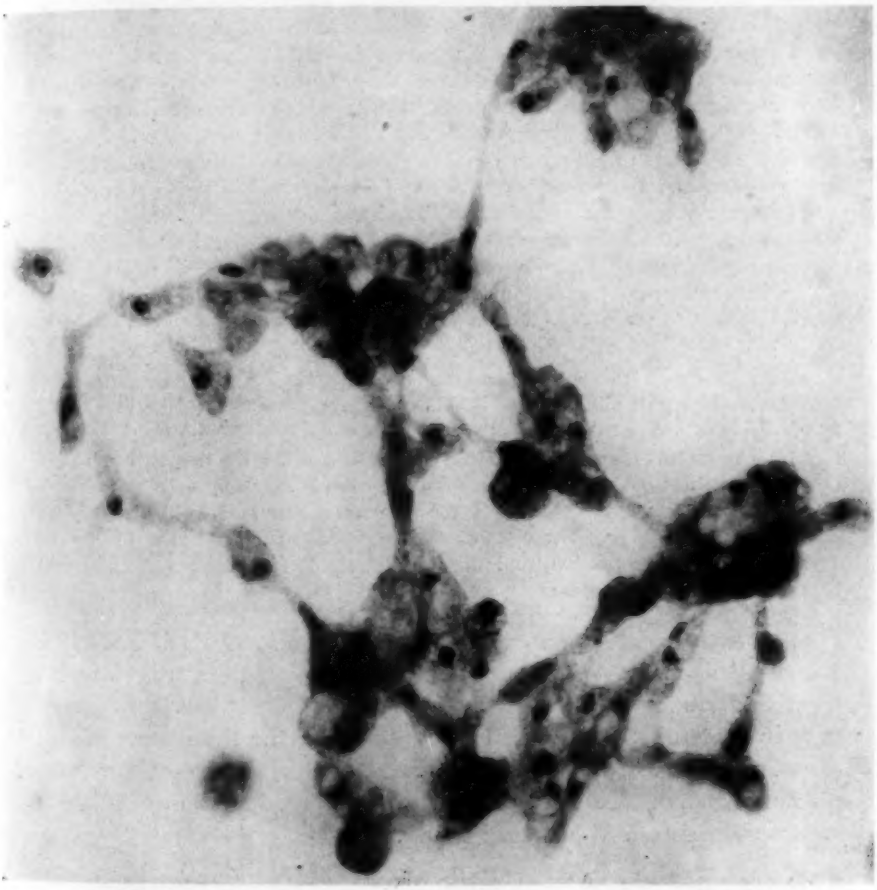

Fig. 4.—Appearance of culture of normal chicken leukocytes after the beginning of its recovery from the effect of inoculation with "free flame" H-37 tuberculin (batch 2); fixed and stained with hematoxylin four days after addition of tuberculin in a dilution of 1:300. Hypertrophied monocytes are shown joining by means of pseudopods; × 520.

cells. The cells showed a pronounced tendency to clump. The clumping occurred by means of pseudopods.

In view of the considerable toxicity of the highest dilution of tuberculin in this experiment, the same batch of tuberculin in still higher dilutions was tested to determine the nontoxic dose.

Protocol 2.—Seven cultures of leukocytes from another normal chicken were made. After twenty-four hours of incubation, the cultures were washed in Tryode solution and patched with plasma. Cultures 1 and 2 kept as controls, and cultures 3, , 5, 6 and 7 each received B. H. tuberculin in dilutions of 1:500, 1:600, 1:700, 1:800 and 1:900. In twenty-four hours and afterward, all the cultures were treated alike and in the usual manner. In twenty-four hours after the addition of tuberculin, cultures 1 and 2 were in good condition. Cultures 3, 4, 5 and 6 revealed numerous dead cells and no migration. Culture 7 was in slightly better condition but considerably worse than the control culture. After three days, cultures 4, 5 and 6 appeared dead and were discarded. On the fifth day, in cultures 3 and 7, some exceedingly long, filament-like cells appeared through the masses of dead cells. These cells were not seen in any of the control cultures of this and previous experiments. On the sixth day after the addition of tuberculin, flasks 3 and 7 represented pure cultures of these cells. The monocytes had transparent and clear protoplasm, without granular material, and they migrated extensively, in chains and groups, throughout the entire clot. Tendency on their part to form a tissue was not observed. They joined by means of pseudopods (correct origin) and occasionally sidewise. These cultures, as well as the controls, in which hypertrophied cells were not seen at any time, remained in good condition for fourteen days. On the fifteenth day, the cells appeared vacuolated. The cultures were discarded.

Protocol 3.—In this experiment, which was similar to those previously described, still higher dilutions of B. H. tuberculin were used. The dilution of 1:1,200 proved somewhat toxic, but the dilution of 1:1,400 did not have any toxic effect. The cultures were observed for seven days after the addition of tuberculin. Some new cells of apparently normal morphology appeared in the flask containing a dilution of 1:1,200. Some dead cells remained in this culture on the seventh day. The culture containing tuberculin in a dilution of 1:1,400 remained in good condition, and the morphology of the cells was similar to that of the control cultures.

The Effect of "Free Flame" H-37 Tuberculin O.T. (Batch 1) on Normal Leukocytes.—The following protocol represents a typical experiment that was repeated several times, in view of the importance attached to it in connection with work to be reported in the third paper of this series. The results of the several trials were perfectly consistent, and the same batch of tuberculin tested in the spring of 1927 showed identical results when retested nine months later.

In this experiment seven leukocytic cultures were used. After twenty-four hours of cultivation, the cultures were in excellent condition. They were washed in Tyrode solution. Patching with plasma was not necessary. One cubic centimeter of Tyrode solution was added to the control cultures 1 and 2. Cultures 3, 4, 5, 6 and 7 received the "free flame" H-37 tuberculin (batch 1) in dilutions of 1:100, 1:200, 1:300, 1:400 and 1:500. In twenty-four hours, cultures 1 and 2 displayed good condition. Culture 3 was entirely dead and it was discarded. There were many dead cells in culture 4. Cultures 5, 6 and 7 were in excellent condition. Four days after the addition of tuberculin culture 4 improved to a certain extent. New, rather elongated monocytes appeared, although the dead cells persisted. The other cultures remained in excellent condition. On the seventh day culture 4 represented a dense growth of hypertrophied, filament-

like, excceedingly long cells, which formed an almost continuous layer. These cells joined by means of distant ends. Areas of elongated monocytes in the form of rosets were found in cultures 6 and 7. The following day, these cultures again assumed a normal appearance. The peculiar appearance of culture 4 remained for the following six days. At this time I decided to determine whether a reversion to normal appearance would occur if the growth were transplanted to a new culture medium. The culture was transplanted. It was in

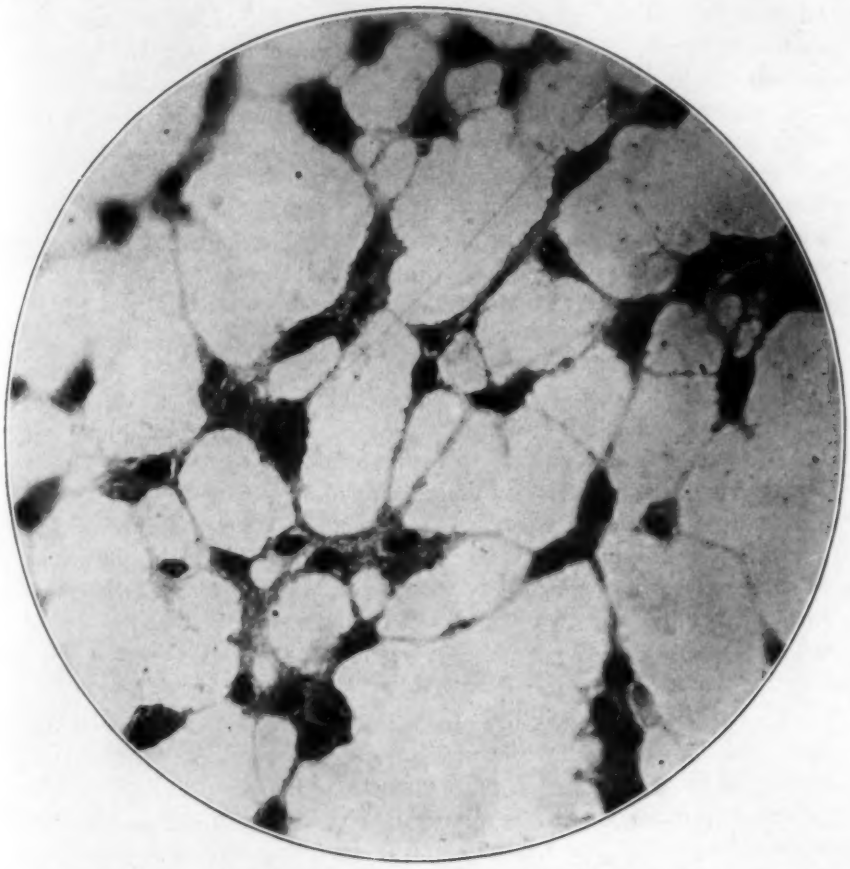

Fig. 5.—Culture of normal chicken leukocytes to which "free flame" H-37 tuberculin (batch 2) in a dilution of 1:300 was added at the ninth day of cultivation; fixed and stained with hematoxylin four days after addition of tuberculin. One may see hypertrophy of monocytes and their joining by means of pseudopods, as described in the text. Further changes in morphology may be seen in figures 6 and 7; × 520.

excellent condition twenty-four hours after the transfer. There was migration into new medium. The lacey appearance of the exceedingly long threadlike cells persisted for the following six days. The culture was then used for another experiment.

The Effect of "Free Flame" and "Water Bath" H-37 Tuberculin (Batch 2), and Avian Tuberculin O.T. on Normal Leukocytes.—These experiments were performed in a manner similar to that of previous experiments. The results can be summarized as follows

Both "free flame" and "water bath" H-37 tuberculin (Batch 2) had a higher toxicity than Batch 1 of this tuberculin. A dilution of 1:300 was somewhat toxic. In twenty-four hours after the addition of this dilution the culture appeared dead. It consisted of large masses of dark and round cells. Beginning with the second day this culture improved. Some monocytes of normal morphology were occasionally seen at the periphery. Definite recovery became evident toward the sixth day; at that time the culture consisted of a dense growth of extremely long, filament-like, anastomosing cells in good condition. A dilution of 1:500 was rather toxic. In twenty-four hours after the addition of this dilution, the number of dead cells was considerably smaller than with tuberculin 1:300. Almost complete recovery was noticed on the third day. Abnormal cells were not seen.

No difference was observed between the effect of "free flame" and "water bath" tuberculin of the same batch.

In several experiments, the avian tuberculin was tested. This tuberculin proved highly toxic in a dilution of 1:1,100. A dilution of 1:1,200 was somewhat toxic. In from forty-eight to seventy-two hours after the addition of a dilution of 1:1,200, many dead cells were noticed. The culture improved, however, in the course of the following few days. The recovery was frequently accompanied by the formation of extremely long, filament-like monocytes, which formed a close network. A dilution of 1:1,400 did not have a toxic effect and abnormal cells were not seen.

COMMENT

Various degrees of toxicity of tuberculin O.T. for normal leukocytes were found to exist. In certain dilutions, there was extreme toxicity, which led to destruction of the monocytes. Recovery did not follow. In other dilutions, there was a mild toxicity, which brought about considerable mortality, but recovery of the surviving cells followed in the course of a few days. The recovery was accompanied by the appearance of certain highly active cells. These were hypertrophied, filament-like, extremely long leukocytes with a pronounced tendency to anastomose by means of distant pseudopods. Definite changes once made understimulation by tuberculin, persisted in the culture in which they were initiated. Reversion to the normal appearance was not observed even on transplantation to a new culture medium. Certain dilutions had no effect on the viability of the cells. However, a slight, possibly accidental effect on the morphology of the cells was sometimes seen with these

dilutions. The cells appeared more elongated than in the control cultures and had an insignificant tendency to arrange themselves in chain or roset formation. Soon a reversion to the normal appearance occurred. Importance was not attached to this possible occurrence of reversible changes in the morphology of cells treated with nontoxic dilutions of tuberculin.

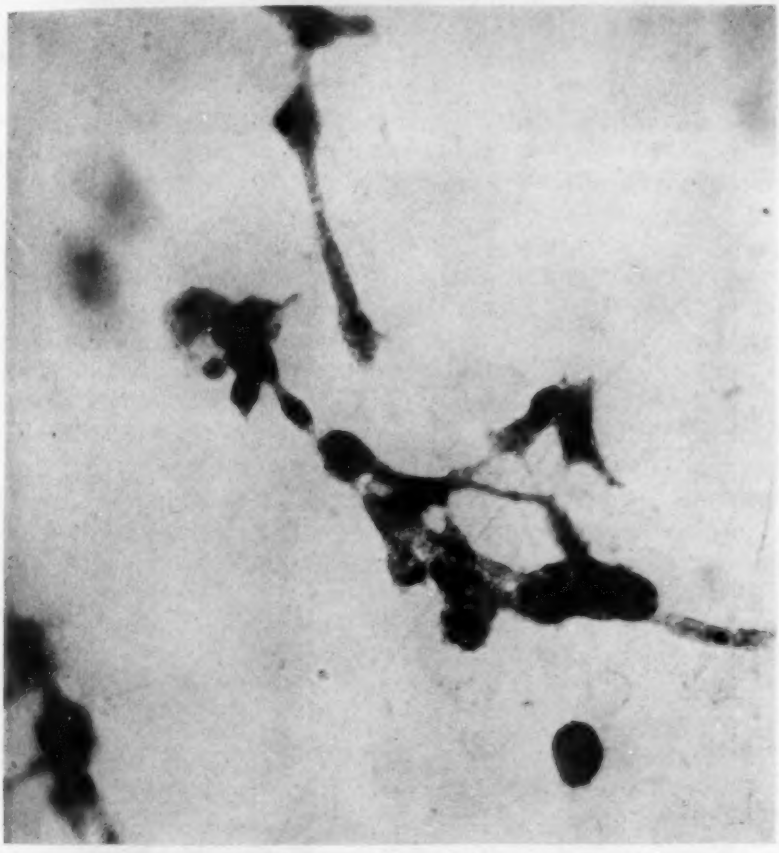

Fig. 6.—Here the hypertrophied monocytes that emerge in leukocytic cultures under the influence of tuberculin are in process of fusing to form giant cells. The final transformation may be seen in figure 7. Hematoxylin; × 520.

It was concluded from these observations that tuberculin is able to destroy monocytes and induce changes in the morphologic appearance of the surviving cells. It remained unsettled whether the change in morphology is correlated with the toxic effect.

Moreover, the observations recorded in this paper brought out the fact that the toxicity of tuberculin varies with the batch employed. The

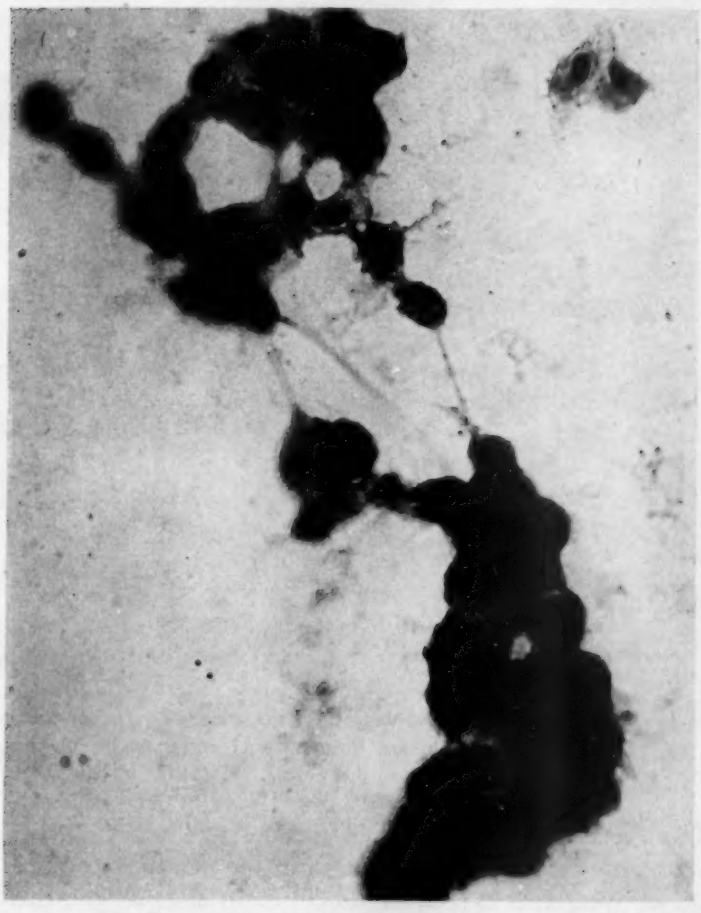

Fig. 7.—Culture of normal chicken leukocytes to which "free flame" H-37 tuberculin (batch 2) in a dilution of 1:300 was added at the ninth day of cultivation; fixed and stained with hematoxylin four days after the addition of tuberculin. Figure 7 with figures 4, 5 and 6 depict progressively the changes in morphology of leukocytes under the influence of tuberculin as described in the text; namely, hypertrophy of monocytes, their joining by means of pseudopods and various stages of formation of giant cells; × 520.

toxic factor was not due to the broth used for preparation of tuberculin, as is seen from the experiments concerned with the effect of glycerin broth on the viability of normal leukocytes. The factor was heat resistant, since there was no difference in the toxicity of "free flame" and "water bath" tuberculin of the same batch.

It was clear that the toxicity was not due to variations of hydrogen ion concentration in the batches employed, since tuberculin diluted in Tyrode solution not less than 1:100 was always of *p*H 7.8 to 8. The toxicity varied to some extent in different batches of tuberculin derived from the same strain and varied considerably in tuberculin from various strains. It suggested that the toxicity of tuberculin depends on the strain employed for the preparation. Conclusive evidence was not obtained, however, to this effect. It is also possible that some other factors were involved.

Observations reported up to this point were made by microscopic examination of unstained cultures. The peculiar effect that the tuberculin had on the morphology of certain cultures necessitated additional examination of stained preparations. The clot was fixed in formaldehyde and stained with hematoxylin or azure blue according to the methods recommended by Fischer.[12] Additional information was gained in this manner.

To culture 59/4 there was added B.H. tuberculin in a dilution of 1:900. During the first few days of cultivation there were numerous dead cells in clumps, but the culture recovered in the course of one week. The microscopic examination of the stained preparation revealed certain peculiarities which were not seen, to any similar degree, in stained preparations of control cultures of monocytes of the same age.

In the tuberculin culture, monocytes of normal appearance were not seen. There were some small lymphocytes. The majority of the monocytes were extremely long and filament-like. Under high power magnification these cells appeared to contain an oval nucleus, which occasionally showed nucleoli. The nucleus was usually darkly stained and, as a rule, appeared homogeneous. Pale nuclei with nucleoli were sometimes observed. This fact will receive consideration in the second paper of this series. The outlines of the cells varied. There were stellate, digitate and fusiform types. The processes were reticulated. Some of the pseudopods were filiform. The monocytes occurred singly or in groups (fig. 4). From the grouping of the cells, giant multinucleated cells originated (fig. 5).

In some instances the multinucleated cells resembled Langhans' giant cells. The origin of the giant cells from the hypertrophied filament-like monocytes was supported by the finding of various transitional stages.

12. Fischer, A.: Tissue Cultures, 1925.

The process of fusion of individual cells and their transformation into single giant cells appeared to be as follows: The hypertrophied cells rarely remained single. As pointed out on many occasions there was a pronounced tendency to clump. After joining by means of the pseudopods and occasionally sidewise, the endoplasm of the anastomosing cells did not fuse at first. The outline and structure of each individual cell could be differentiated in such groups. However, gradual shortening or "drawing in" of the anastomosing processes occurred later. There were seen fusing cells in which the nuclei of the cells came nearer to each other than at first and the anastomosing processes disappeared altogether at the point of junction. Since each cell joined the others by means of one pseudopod, the outline of the free processes persisted in some of the multinucleated cells. However, these processes disappeared in such cells, since perfectly round giant cells were seen. The nuclei were located at the periphery of the giant cells. This location of the nuclei was due, probably, to the fact that the cells joined by means of the distant ends of the pseudopods with the nuclei located at the opposite ends of the cells. In many instances, the nuclei were displaced toward the center of the giant cells.

In addition to these cells other cells of different morphology were seen. The latter were round and large, although they varied in size. The protoplasm was pale, granular and frequently vacuolated. The nucleus was darkly stained, round or irregular and located mostly eccentrically. Often they contained single vacuoles of considerable size. The cells were seen singly and in groups of two. They were interpreted as epithelioid cells. Similar cells were occasionally seen in stained preparations of old normal leukocytic cultures.

It appeared from these observations that tuberculin was able to induce peculiar morphologic changes in vitro. The changes consisted of the appearance of hypertrophied filament-like monocytes. Their main characteristic was their ability to fuse and form giant cells. Incidentally, cells resembling epithelioid cells were seen in large numbers.[13] These changes were considered characteristic of the histology of the tubercle in vivo.

The formation of tubercle in vivo under the influence of dead tubercle bacilli and that of tuberculin has been thoroughly investigated by different workers. The literature on the subject was discussed by Sabin and Doan [14] in a recent paper. They definitely established the possibility of reproducing histologically typical tuber-

13. The histogenesis of these cells and their relation to the hypertrophied filament-like monocytes was not investigated, since the problem did not belong to the scope of the work embodied in this series.

14. Sabin, F. R., and Doan, C. A.: J. Exper. Med. **46**: 645, 1927.

culous elements by means of a certain fraction of tubercle bacilli in the peritoneal cavity of the rabbit. My observations in this part of the present paper tend to show that tuberculin is able to induce tubercle formation in explanted leukocytes. I purpose to bring further support to this belief by comparative studies of tuberculin cultures and cultures infected in vitro by live tubercle bacilli under the same experimental conditions.

CONCLUSIONS AND SUMMARY

In order to initiate studies on the immunologic reactions of tuberculosis in vitro it was necessary to determine first the effect of tuberculin on normal tissues. Leukocytes were believed to be best suited for this purpose. Certain methods of cultivation described by Carrel were chosen, since they allowed prolonged observations under conditions of uninterrupted growth and gave consistent and reliable results. Various batches of tuberculin were tested. Definite criteria for determining the effect of tuberculin were put forth. The observations established the following:

Tuberculin possesses in vitro a toxicity for normal leukocytes. The toxic factor is heat resistant. The significance of this factor is still to be determined; work on this point is in progress. Its relation to factors toxic for the whole animal found by earlier writers,[14] should also be determined.

Coincidentally, tuberculin is able to influence to a great extent the morphologic appearance of the monocytes. Definite changes once made under stimulation by tuberculin persist in the culture in which they are initiated. Moreover, reversion to the normal appearance is not observed even on transplantation to a new culture medium.

The changes induced are characteristic of tuberculosis. It is believed that it is possible to reproduce the tubercle in vitro by means of tuberculin.

The second paper in this series embodies an analysis of the effect of tuberculin on the morphology of normal leukocytes.

IMMUNOLOGIC REACTIONS IN TISSUE CULTURES

II. ANALYSIS OF THE EFFECT OF TUBERCULIN O. T. ON THE MORPHOLOGY OF NORMAL LEUKOCYTES IN VITRO*

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In a previous report I [1] showed that tuberculin is toxic for normal leukocytes and is able to induce in them certain morphologic changes characteristic of tuberculosis. The work reported in this paper had in view: (1) a comparison of the morphologic changes in tuberculin-treated cultures of leukocytes with those brought about through stimulation of leukocytes by live tubercle bacilli under similar experimental conditions; and (2) a determination of the optimal conditions for inducing morphologic changes under the influence of tuberculin.

METHODS

The same methods were employed in this work as were used in the work described in the first paper of this series.

To obtain tuberculous cultures of leukocytes a small clump of tubercle bacilli [2] was deposited next to the blood fragment before the coagulation of the culture medium occurred. In the majority of instances the bacillary clumps remained in actual contact with the explanted tissue. The tubercle bacilli multiplied with ease in this medium; after from seven to ten days new bacillary colonies appeared in the vicinity of the original inoculum and, later, over the entire flask.

EXPERIMENTS

I. *The Effect of Live Tubercle Bacilli on Cultures of Normal Leucocytes.*—The effect of tubercle bacilli on explanted tissues has been studied by several authors. Pure cultures of blood leukocytes infected with tubercle bacilli have been studied by Maximow.[3] In my experiments the inoculated leukocytic cultures were kept growing for varying periods, up to seven days. After every second day they were transplanted into fresh medium. Rabbit's leukocytes were employed.

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* From the laboratories of the Mount Sinai Hospital.

* This work was aided by a grant from Eugene Meyer through the Manhattan Research Foundation.

1. Shwartzman, Gregory: Immunologic Reactions in Tissue Cultures: I. The Effect of Tuberculin O. T. on Normal Leukocytes in Vitro, Arch. Path., this issue, p. 773.

2. Moist and abundant cultures not over two months old were employed in the experiments.

3. Maximow, A. A.: J. Infect. Dis. **37**:418, 1925.

To allow comparison of the morphologic changes produced in cultures by tuberculin with those produced by live tubercle bacilli I employed methods different from Maximow's. Chicken leukocytes were cultured in Carrel D_5 flasks. With this method transplants were not necessary and observations could be made for three weeks and sometimes longer. The results were uniform, little variation in the process of change being noticed in several cultures of normal leukocytes. The process of change was as follows:

A clump of tubercle bacilli, when deposited as close as possible to the fragment of leukocytes and embedded in the medium, became surrounded by migrating leukocytes in the course of the first twenty-four hours of incubation. When, however, the bacillary clump was deposited at some distance from the blood fragment, the migrating cells, instead of forming the usual circular area around the explant, directed themselves toward the clump. Frequently, therefore, the migrating area assumed an oval shape, elongated toward the point where the bacillary clump was embedded.

The immediate effect of the bacilli was toxic. The leukocytes located about the bacillary clumps were killed off, as was evidenced by the presence of masses of dark and highly granulated leukocytes. However, in a different plane of the same area or among the clusters of the dead cells it was frequently possible to observe slightly elongated ameboid cells with homogeneous protoplasm. After twenty-four hours of incubation, and later, the areas at some distance from the bacillary clumps consisted of a rather dense layer of active, morphologically normal monocytes. The growth of these cells located at some distance from the bacillary colonies was undoubtedly being stimulated. In fact, the area of migration increased steadily and became larger and denser than that of normal cultures of the same leukocytes. This stimulation was observed in cultures many times. It appeared, therefore, that in the course of from twenty-four to forty-eight hours of close contact with normal leukocytes tubercle bacilli, if in large numbers, were toxic for the leukocytes; but that when the leukocytes were at a distance or in areas where there were only few bacilli, the bacilli served to stimulate their power of migration. The cells of normal appearance persisted in some areas for a considerable length of time. The bacilli grew in the same cultures. In the course of the first week, new bacillary colonies appeared at various points of the clot.

Toward the end of the first week of incubation, interesting modifications in the morphology of leukocytes became evident. Not only were there persisting areas of normal monocytes in several places—especially, close to the bacillary clumps—but these monocytes enlarged and transformed themselves into extremely long digitate, stellate and filiform cells.

These long cells showed a definite tendency to clump as soon as they appeared in appreciable numbers, namely, from the eighth to the tenth day of incubation. The lacy appearance of a culture containing these cells was a highly conspicuous feature of cultures infected with tubercle bacilli. The hypertrophied cells, once having appeared in a culture,

Fig. 1.—Leukocytes inoculated with tuberculin H-37 and incubated for nineteen days. The culture has maintained this appearance since the seventh day. Filament-like monocytes are seen showing a tendency to anastomose by means of pseudopods. Stained with hematoxylin; × 520.

continued to multiply during the entire second week and not infrequently also during the third week of incubation. They were transparent and clear, showing little evidence of degeneration, and appeared throughout the entire medium. Their ability to grow in close proximity

to bacillary clumps and to appear among the masses of cells killed by tubercle bacilli suggested a definite resistance to the toxicity of tubercle bacilli. This point should, however, receive special consideration.

The cells in clusters represented transitional stages of Langhans' giant cells, as is seen from stained preparations. The process of fusion seemed to be identical with that described in connection with the formation of giant cells in cultures treated with tuberculin.[1] The description is therefore omitted here.

At the beginning of the second week of cultivation, rather large spherical cells with hypertrophied nuclei and vacuolated protoplasm

Fig. 2.—Leukocytes inoculated with tuberculin H-37 and incubated for thirteen days. This pictures a living culture in which anastomosing filament-like monocytes have appeared; × 100.

appeared in the cultures. In stained preparations, their morphologic structure was identical with that of the epithelioid cells observed in the cultures treated with tuberculin. The number of these cells persistently increased. They aggregated in dense masses and formed films over large areas. Toward the end of the second week, the epithelioid cells underwent rapid degeneration. They became extremely large, highly granulated and vacuolated, the nuclei enlarged enormously, and finally the cells died. These degenerated cells represented Smyth's "shadow cells."

The morphologically normal monocytes disappeared during the third week of cultivation.

A comparison of the process described here, with Maximow's observation of leukocytic cultures infected with live tubercle bacilli, discloses one point of difference. Maximow observed that after five or six days the monocytes enlarged enormously. These cells were scattered

Fig. 3.—Giant cells in a leukocytic culture inoculated with tuberculin H-37 and incubated for seventeen days. Stained with hematoxylin; × 260.

in the plasma medium singly or in small groups, and here they displayed their ameboid character. They were found in large numbers in the interior of the explanted fragment, also, where they were often assembled in compact masses.

In my observations the hypertrophied monocytes that were scattered singly or in small groups toward the end of the first week, during the

Fig. 4.—Same culture as shown in figure 3; × 260.

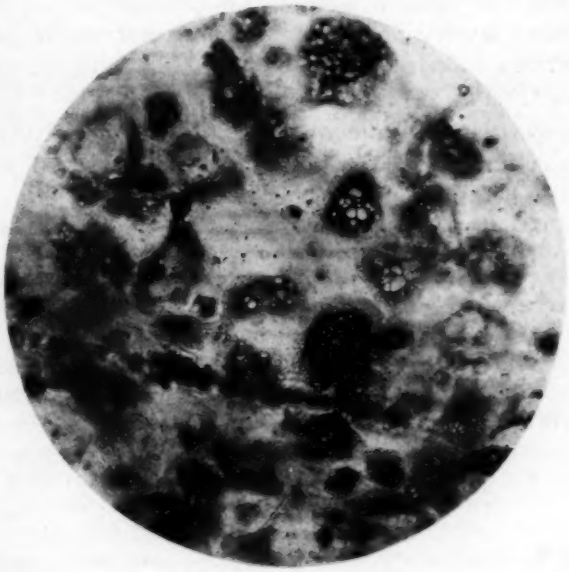

Fig. 5.—Leukocytes inoculated with tuberculin H-37 and incubated for nine days. "Shadow" cells are seen. Stained with hematoxylin; × 100.

second week of cultivation appeared conspicuously as a network of cells in many areas and often throughout the entire flask. The appearance of numerous anastomosing filament-like cells is of great help in recognizing the transformation of explanted normal leukocytes into a culture typical of tuberculosis, especially, when examined in unstained condition. In fact, this picture never failed to make its appearance in tuberculous cultures.

The difference pointed out was most likely due to the fact that Maximow did not study the cultures longer than seven days.

The comparison of the changes in tuberculous cultures with those observed in cultures treated with tuberculin, cultivated under the same conditions and studied for similar periods, disclosed the following points of similarity and dissimilarity:

Both tuberculin and live tubercle bacilli had a definitely toxic effect on normal leukocytes in vitro.

The appearance of hypertrophied, filament-like monocytes that had a tendency to anastomose was an outcome necessary to coincident improvement in the condition of cultures.

Under both conditions the filament-like elements in clusters appeared at the beginning of the second week and thereafter multiplied persistently until there was a peculiar network, which spread widely.

In cultures treated with tuberculin, the process was of less severity than in cultures infected with live tubercle bacilli. Caseation and formation of "shadow cells" were not seen.

The comparison of both processes demonstrates that changes observed in cultures treated with tuberculin are essentially similar to those produced by means of live tubercle bacilli.

EXPERIMENTS

II. *The Effect of Various Batches of Tuberculin on the Morphology of Explanted Leukocytes.*—A series of experiments was performed to determine the optimal conditions for inducing morphologic changes in explanted leukocytes by means of tuberculin O. T. The following protocols represent experiments to determine whether the batches of tuberculin used for inoculation differed in power to induce changes in leukocytic morphology.

Protocol 1.—A culture of leukocytes (54/4),[4] twenty-four hours old, received 1 cc. of B. H. tuberculin in a dilution of 1 : 500. This dilution of tuberculin was highly toxic. Twenty-four hours after it was added, few living cells were seen. Dead cells persisted for seven days. On the fifth day, a few filiform monocytes appeared among the clumps of dead cells. On the sixth day, the culture assumed

4. The numerator indicates the number of the experiment; the denominator represents the number of the culture.

the typical appearance of a tuberculous culture. I did not observe any reversion to a normal appearance during the following two days.

PROTOCOL 2.—A culture of leukocytes (55/6), twenty-four hours old, received 1 cc. of B. H. tuberculin in a dilution of 1:700. In twenty-four hours, there were many dead cells and a few living monocytes. Toward the fifth day, the culture showed signs of improvement. Nine days after the addition of tuberculin, the culture contained a dense growth of extremely thin and long anastomosing cells. I did not observe any reversion to a normal appearance during the following eighteen days.

PROTOCOL 3.—Two twenty-four hour old cultures (59/8 and 59/9) received each 1 cc. of B. H. tuberculin in dilutions of 1:500 and 1:900, respectively. Twenty-four hours after the addition of tuberculin one culture (59/8) contained numerous dead cells. There were some dead cells in the other (59/9). At the end of forty-eight hours the first culture (59/8) remained in the same condition. The second culture (59/9) showed more dead cells than on the previous day. Changes in morphology were first noticed on the fifth day after the addition of tuberculin. The dead cells were present as well. On the seventh day, both cultures assumed a typical appearance of tuberculous leukocytes. I did not find any reversion to a normal appearance in the course of the following eight days.

PROTOCOL 4.—Three twenty-four hour old cultures (60/6, 60/7 and 60/8) received B. H. tuberculin in dilutions of 1:1,000, 1:1,200 and 1:1,400. Twenty-four hours after the addition of tuberculin, there were some dead cells in culture 60/6. Dead cells were not found in cultures 60/7 and 60/8. Culture 60/6 improved in twenty-four hours. Cultures 60/7 and 60/8 remained in good condition during the rest of the period of observation. I did not see any abnormal cells at any time. Observations were made for eight days after the addition of tuberculin.

PROTOCOL 5.—This protocol represents a summary of cultures of leukocytes similarly treated but derived from different chickens. Twenty-four hour old cultures 68/1, 70/2, 75/2, 84/2 and 88/2 received each 1 cc. of tuberculin H-37, "free flame" batch 2, in a dilution of 1:300. Twenty-four hour old culture 88/1 received 1 cc. of the same tuberculin in a dilution of 1:200. Culture 68/1 contained numerous dead cells in clumps twenty-four hours after addition of tuberculin. The following day there was marked improvement. Many new active cells of normal morphology appeared at the periphery. Five days after the addition of tuberculin the culture was in excellent condition. Abnormal cells were not seen in this culture until it was nine days old. The culture was then used for another purpose. Cultures 70/2 and 75/1 behaved in a manner identical with that of culture 68/1.

Tuberculin batch 2 in a dilution of 1:300 was also toxic for culture 84/2. On the third day after the addition of tuberculin there was improvement. On the eighth day the culture assumed the typical appearance of a tuberculous culture. I did not see any reversion to a normal appearance in the course of the following four days.

The fate of culture 88/2 was identical with that of culture 84/2. Culture 88/1 contained numerous dead cells for forty-eight hours after the addition of tuberculin; then gradually the dead cells were replaced by hypertrophied monocytes, which began to anastomose and appeared in large numbers on the eighth day after addition of tuberculin. I did not find any reversion to a normal appearance in this culture.

PROTOCOL 6.—Three twenty-four hour old cultures (55/9, 55/10 and 55/11) received each 1 cc. of "free flame" tuberculin H-37, batch 1, in dilutions of 1:200, 1:300 and 1:400, respectively. Some toxicity was revealed by the first dilution in twenty-four hours. Higher dilutions showed no toxicity. Culture 55/9 recovered in two days. On the eighth day after the addition of tuberculin, this culture assumed the typical appearance of a tuberculous culture. Changes were not observable in the morphology of cultures 55/10 and 55/11. Observations were made until the cultures were two weeks old.

Identical results were obtained with another series of cultures derived from another chicken.

Conclusions from Experiments Made.—The ability of tuberculin to produce morphologic changes in explanted leukocytes varied with the batch employed. There was not any significant variation in this property in two batches of tuberculin prepared from the same strain (H-37). There was, however, a definite difference in tuberculin derived from another strain (105). Higher dilutions produced these changes invariably. It was suggestive that this ability of tuberculin depends on the strain employed for the preparation. Conclusive evidence was not available, however, to support this view. It was also possible that the variations were due to other unrecognized factors.

In the majority of instances, the toxicity of tuberculin was coincident with the morphologic changes. However, in two experiments with the second batch of "free flame" H-37 tuberculin, the dilution of 1:300 was invariably and definitely toxic but it did not induce morphologic changes. The relation of toxicity to the morphologic changes will be discussed again.

As may be seen from the protocols, the tuberculin was added to twenty-four hour old leukocyte cultures. The morphologic changes typical of tuberculosis always occurred after a certain interval, which varied from five to nine days. It was assumed that the observed period of incubation was necessary because of the leukocytic cultures having to reach a certain age to become susceptible to the properties of tuberculin that induce morphologic changes. If this assumption is correct, the changes should take place quicker in aged cultures. The following experiments were made to investigate this assumption.

The Effect of Tuberculin "Free Flame" H-37, Batch 2, on the Morphology of Explanted Normal Leukocytes of Different Ages.—In determining whether the aged cultures of normal leukocytes would undergo morphologic changes under the influence of tuberculin quicker than twenty-four hour old cells, H-37 tuberculin (batch 2) was employed. As may be seen from previous experiments, this tuberculin had an uncertain effect on the morphology of twenty-four hour old leukocytes when added in a dilution of 1:300.

PROTOCOL 1.—A series of leukocytic cultures from normal chicken's blood was made and incubated for twenty-four hours. Following this period of incubation the cultures were in good condition. The area of migration consisted of numerous polymorphonuclear neutrophil leukocytes and some small lymphocytes. Only a few large lymphocytes were seen at the periphery. One twenty-four hour old culture received "free flame" H-37 tuberculin (batch 2) in a dilution of 1:300. A control culture received 1 cc. of Tyrode solution. After the next twenty-four hours, all the cultures were washed in Tyrode solution, and a mixture of serum, Tyrode solution and embryonic extract was added. The microscopic examination of the culture which had received tuberculin was made daily for twelve days thereafter, and comparison was always made with other normal cultures of the same series. In twenty-four hours after addition of tuberculin, the polymorphonuclear leukocytes were dark, granular, round and small. The monocytes were of similar appearance. Control cultures were in excellent condition. Later, the tuberculin culture began to show improvement. New monocytes gradually appeared at the periphery, and toward the fourth day were in good condition. In fact, there was not any difference between the condition of this culture and that of the control cultures at this time. Improvement continued. I did not see any definite changes in the morphology of the cells at any time of observation in the course of twelve days. Occasionally, elongated cells in groups were seen, but the following day there was reversion to normal appearance. Other cultures of this series received H-37 tuberculin (batch 2) in a dilution of 1:300 at the age of three, six and nine days. Since the three day old leukocyte culture contained only a few polymorphonuclear neutrophil leukocytes, it was impossible to determine the effect of the tuberculin on these cells. Tuberculin, however, was toxic for the monocytes of this age. After twenty-four hours of contact with tuberculin the culture revealed numerous dead cells with a few living monocytes, which appeared hypertrophied and active. The culture was washed in Tyrode solution and to it was added a mixture of serum, Tyrode solution and embryonic juice, but it did not improve in the next twenty-four hours. However, toward the fourth day it was observed to be improving, and finally, on the fifth day after addition of tuberculin, the culture was considered of normal appearance. I did not see any definite morphologic changes in this culture at any time during the period of observation.

The fate of the culture to which tuberculin was added at the age of six days was different. The tuberculin was decidedly toxic for this culture after twenty-four hours. There were numerous dead cells in clumps. During the second twenty-four hours, a complete change occurred in the leukocytic morphology. Through the masses of dead cells, numerous extremely thin and long cells appeared. These cells soon formed a close network in many areas. Various stages of fusing were seen. The culture appeared to be of a morphology typical of tuberculous cultures. The culture remained in this condition until used for another purpose on the twelfth day of cultivation.

The nine day old culture responded similarly to tuberculin. The latter in a dilution of 1:300 was again definitely toxic. The culture recovered in from two to three days. In forty-eight hours after addition of tuberculin, it assumed the typical appearance of a tuberculous culture.

As may be seen from these experiments, there was no difference in toxicity of the same tuberculin for cultures of different ages.

It was also demonstrated that aged leukocyte cultures underwent definite morphologic changes typical of tuberculosis within forty-eight

hours after the addition of tuberculin. The promptness of the changes was in contrast with observations made before on young cultures treated with the same dilution of this tuberculin. In these young cultures the changes occurred only occasionally, and when they did occur an incubation period of from seven to nine days was required. It may be stated, therefore, that the cultures have to reach a certain age before they can undergo the described changes.

It was of interest to determine whether the aged cells not only undergo these changes quicker than young ones but also are more susceptible to this property of tuberculin. For this purpose a dose of tuberculin about five times weaker than one which is necessary to produce the changes in a young culture was employed.

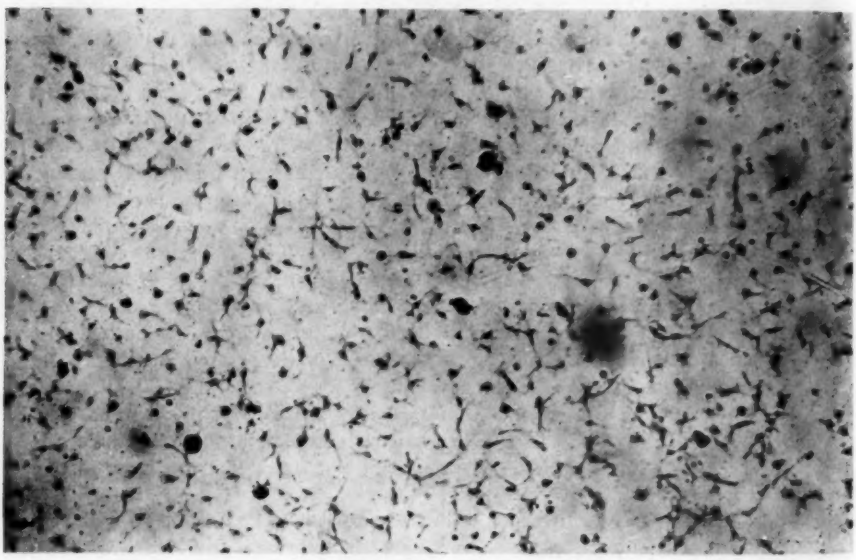

Fig. 6.—A leukocytic culture five days old. It shows a spontaneous tendency to formation of elongated monocytes in chains. Stained with hematoxylin; × 100.

To a leukocytic culture seven days old "free flame" H-37 tuberculin (batch 2) wsa added in a dilution of 1 : 1,000. I did not see any toxic effect in twenty-four hours after addition of tuberculin nor thereafter. In forty-eight hours, there was a complete change in morphology, giving a picture characteristic of tuberculosis. These observations were repeated a few times with identical results.

In summary, it may be stated that the aged leukocytes in vitro which undergo readily morphologic changes under the influence of tuberculin are also more susceptible to such influence than young cultures.

Although no special experiments were performed to throw light on the cause of this difference in response between aged and young leukocytic cultures, nevertheless, certain possibilities can be discussed.

The first possibility is that there are more cells which are apt to undergo these changes in the aged cultures, and therefore the process of transformation goes on more quickly. In fact, the number of monocytes steadily increases as the age of the culture advances. If this explanation is correct, the following process should be observed:

The changes should begin at once, since monocytes are present from the beginning of cultivation. Once the changes begin, they should

Fig. 7.—Same culture as shown in figure 6; × 520.

progress, as is seen from previous observations on cultures containing the changed elements. They should also develop fully about the time when the normal culture usually contains a large number of monocytes. If the tuberculin is toxic and kills off some monocytes, then higher dilutions should be more favorable for the development of the typical changes. Moreover, a young culture should eventually be changed by

means of approximately the same amount of tuberculin as an aged culture. The process may possibly be slower. However, the analysis of the conditions necessary for the occurrence of the morphologic changes in cultures treated with tuberculin brings out certain points against this explanation.

Although it is possible that the changes begin at once and unnoticeably in young cultures, they do not develop progressively. After a certain latent period there is a sudden change in morphology at a proper time.

The incubation period, also, frequently exceeds the time when the control normal cultures already contain numerous monocytes.

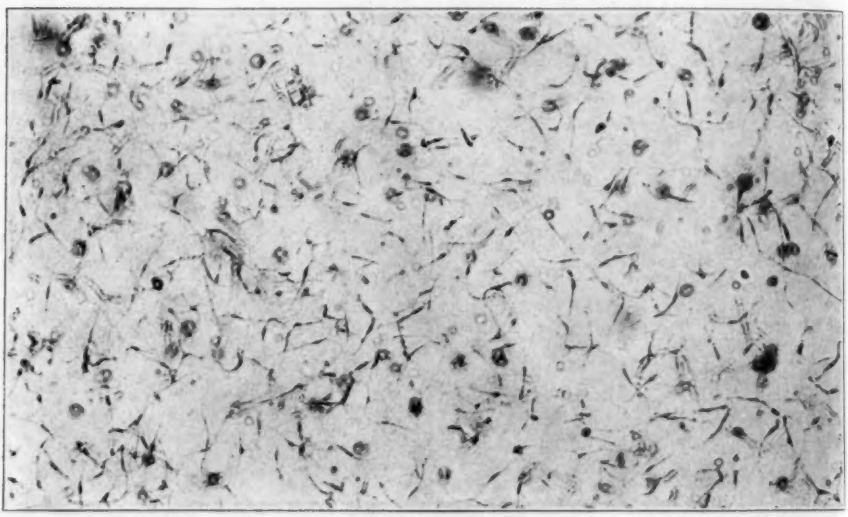

Fig. 8.—This culture showed a spontaneous tendency to the formation of elongated monocytes in chains. On the seventh day of cultivation the culture appeared normal again. On the tenth day of cultivation, "free flame" H-37 tuberculin (batch 2) in a dilution of 1:1,000 was added. The culture was fixed and stained on the fourth day after the addition of tuberculin; × 100. Figures 6, 7 and 8 bring out the difference between leukocytic changes springing from a spontaneous tendency to formation of elongated anastomosing monocytes and the changes obtained by means of inoculation with a high dilution of tuberculin.

Moreover, in some experiments in which the amount of tuberculin added to a young culture is not too toxic, a considerable number of what appear to be normal monocytes in good condition is present during the latent period.

In addition, more concentrated amounts of tuberculin are necessary to obtain changes in young cultures, as compared with aged cultures.

It appears, therefore, that another explanation has to be sought. At present it is assumed that the aged monocytes undergo certain biologic

changes in the process of cultivation, and that these changes enable them to transform themselves into tuberculous elements on proper stimulation.

The tuberculin added to the culture is probably absorbed by the clot and possibly also by the cells. There it stimulates the proper changes as soon as the cells are ready for it.

Fig. 9.—A culture of fibroblasts stained with azure blue. The appearance of nuclei with nucleoli and a tendency to formation of tissue should be noted; × 520.

To complete these studies on the optimal conditions for inducing the morphologic changes, I sought to determine whether leukocytes derived from different normal chickens vary in their ability to undergo these changes.

Occasionally, in the course of the work, series of leukocytic cultures were obtained which appeared somewhat peculiar. The monocytes were elongated and frequently filament-like and had a variable tendency to

grow in small groups and in chains. The larger monocytes were located close to the original explant, while the normal monocytes were located at the periphery. This peculiar morphology was noticed in young cultures. Gradually thereafter the sets would resume normal appearance—this taking place from about the eighth to the tenth day of cultivation. These pecularities were considered somewhat similar to those described previously by Carrel [5] in his experiments on differentiation of monocytes into fibroblasts.

Variation in Susceptibility of Explanted Leukocytes Toward the Morphology-Changing Properties of Tuberculin.—In studies on variations in susceptibility to the morphology-changing effect of tuberculin both the sets of normal leukocytes and those showing atypical growth

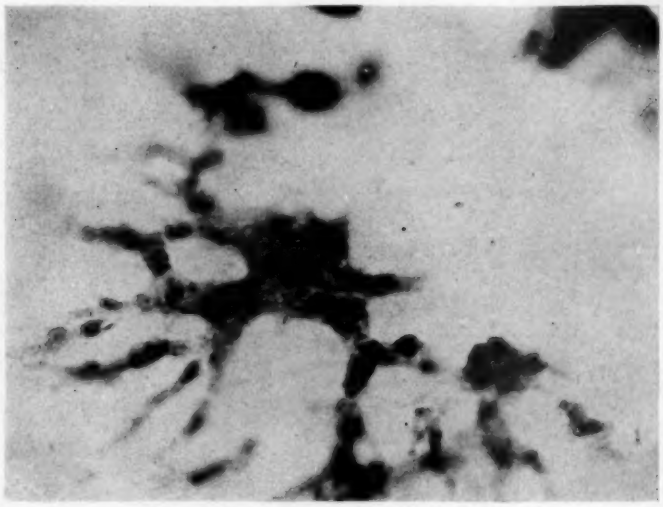

Fig. 10.—Leukocytes treated with tuberculin. Stained with azure blue. The nuclei are dark. Occasional nucleoli may be seen. The cells join sidewise and by means of pseudopods; × 520.

were included. Various batches of tuberculin were titrated in aged leukocytic cultures, as follows:

Protocol 1.—A series of normal leukocytic cultures was treated in the usual manner and not used for any experimental purpose until the ninth day. On the third day of incubation in many cultures of this series, elongated and also filament-like cells appeared in different areas next to the original blood fragment. In unstained preparations these cells resembled somewhat the filiform hypertrophied monocytes of tuberculous cultures. However, the lacy appearance was not shown, and the clumping was in the form of chains and rosets. The periphery consisted of normal monocytes. On the seventh day of cultivation, few hypertrophied monocytes were seen. On the eighth day, the cultures appeared perfectly normal.

5. Carrel, A.: J. Exper. Med. **36**:365, 1922.

To some of these cultures, "free flame" H-37 tuberculin (batch 2) in a dilution of 1:1,100 and B. H. tuberculin in a dilution of 1:1,200 were added on the ninth day of incubation. The remaining four cultures were kept as controls. In twenty-four hours after the addition of tuberculin, the tuberculin cultures consisted almost entirely of extremely thin and long monocytes, which formed a close network. The appearance was typical of tuberculous cultures. This appearance persisted to the end of the period of observation, namely, from about eight to ten days. The four control cultures did not show any abnormal appearance during this time.

PROTOCOL 2.—To a set of normal leukocytes which did not show any abnormalities at any time of observation, various batches of tuberculin in high dilutions (in the same dilutions as were used in the experiment reported in protocol 1) were added on the eighth day of cultivation. Twenty-four hours after the addition of tuberculin, I did not observe any morphologic changes. In forty-eight hours, all the tuberculin cultures had become filament-like monocytes, scattered singly or in small anastomosing groups. Three days after the addition of tuberculin, the majority of these cells had disappeared, and the cultures had reverted to their normal appearance.

As is clear from these observations, there was for normal leukocytes in aged cultures a definite variation in susceptibility to the morphology-changing properties of tuberculin. Cultures that showed a spontaneous but reversible tendency to the formation of anastomosing and filament-like monocytes were considerably more susceptible to this effect of tuberculin than normal monocytes. It should be pointed out that their susceptibility was tested at a time when abnormal cells had not yet been seen. The change to abnormal appearance was not observed in four control cultures of the same set.

All the cultures which were described in the foregoing protocols as normal ones did not show the peculiarities described in this experiment.

The abnormal cultures that were highly susceptible to the effect of tuberculin were not studied from the point of view of differentiation, since they soon assumed the normal appearance. The fact of their abnormality is recorded here simply by way of pointing out a possible source of confusion in the fact that variations in susceptibility of explanted leukocytes to the morphology-changing properties of tuberculin can occur. It remains yet to determine whether the tendency of a culture to differentiation has any relation to its ability to undergo the typical changes under the influence of tubercle bacilli and tuberculin.

In addition, I ought to mention that, in the high dilutions of H-37 tuberculin (batch 2) employed, I did not observe any toxicity. Nevertheless, these dilutions induced the usual morphologic changes. It may seem, therefore, that the toxicity of tuberculin has no relation to the changes induced in leukocytic morphology by tuberculin. This observation is not, however, conclusive, since the toxic effect could be slight and escape observation.

CONCLUSIONS AND SUMMARY

The effect of tuberculin on the morphology of explanted leukocytes was compared with the changes produced in leukocytes infected with tubercle bacilli under the same experimental conditions. Both the tuberculin and the live tubercle bacilli had a similar effect on the morphologic appearance of these cultures.

The ability of tuberculin to stimulate these changes varied with the batch employed. In different preparations with tuberculin of the same strain little variation showed, but in preparations of different strains, there was a considerable difference in ability to stimulate changes.

When added to a young culture, tuberculin stimulated morphologic changes after a certain period of incubation. It took effect more quickly when added to aged leukocytic cultures. Moreover, higher dilutions of tuberculin produced the changes in older cultures. It was concluded that aged leukocytic cultures undergo the changes more readily and are more susceptible than recently explanted leukocytes.

There were variations in the susceptibility of aged leukocytes to the effect of tuberculin. The cultures which showed certain reversible abnormalities were more susceptible to this effect than normal cultures.

The morphologic changes were independent of the toxic effect of tuberculin, since in certain dilutions which brought about the changes there was not noticeable toxicity. It is possible that a slight toxicity escaped observation.

Work is under progress to determine the effect of tuberculin on leukocytes derived from tuberculous chickens.

PENETRATION OF THE HEART BY AN ALUMINUM PIPESTEM *

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Reports of stab, gunshot and other wounds of the heart are frequently encountered in the literature. In 1909, Peck [1] was able to collect from the records of surgery 161 cases. In 1912, Pool [2] amended Peck's list, adding seventy-nine further instances. More recently, Smith [3] tabulated fifty-eight cases exclusive of two seen in his own practice.

Less common, but not rare, are descriptions of foreign bodies in the heart or pericardium. Among the widely various objects which have been found may be mentioned bullets, shell fragments, needles, pins, nails, toothpicks, fish bones and particles of clothing.

An instance of death resulting from a foreign body in the heart which came to our attention recently presents several interesting features, chief of which are the unusual means of suicide, the nature of the foreign object, the difficulty of locating the foreign body by means of the roentgen ray and the duration of life following the injury. These features we feel are of sufficient interest to warrant recording the case.

Thorough search of the available literature on cardiac injuries has failed to disclose an identical instance. The only similar case is that reported by Davenport [4] of suicide attempted by plunging a 16 penny casing nail, 3½ inches in length, through the right ventricle. The accident occurred in a hospital and prompt surgical intervention saved the patient's life.

REPORT OF A CASE

History.—R. M., a man, aged 47, a mechanic, when first seen by Dr. Staub in the early part of June, 1928, was in the depressed phase of manic-depressive psychosis, which had developed following a prolonged period of hospitalization

* From the Department of Pathology, University of Oregon Medical School.

1. Peck, Charles H.: The Operative Treatment of Heart Wounds, Ann. Surg. **50**:100, 1909.

2. Pool, Eugene H.: Treatment of Heart Wounds, Ann. Surg. **55**:485, 1912.

3. Smith, William Randolph: Cardiorrhaphy in Acute Injuries, Ann. Surg. **78**:696, 1923.

4. Davenport, George L.: Suture of Wound of the Heart, J. A. M. A. **82**: 1840 (June 7) 1924.

for pleurisy with effusion. He had been told that this was probably of tuberculous origin.

Suicidal tendencies soon became manifest, and close observation was instituted. On June 10, while still at home, he attempted suicide by jabbing the blade of a small penknife into the pharyngeal wall, but without serious injury. He also told relatives that he had swallowed one or two pocket handkerchiefs in order to produce suffocation. On the same day, he informed the family that, during the momentary absence of relatives from the room, he had plunged the aluminum tube of his pipestem through the left wall of the chest, completing the ingress of the tube by pressure on it with the rubber mouthpiece.

When seen by Dr. Staub a short time after the accident, the patient was in good physical condition, and the only evidence to support his story was the presence of three small wounds in the skin at the level of the sixth left interspace in the nipple line. There was no bleeding from the wounds and no evidence of internal hemorrhage. Because the nature of the foreign body was known, a roentgen-ray study was not made at this time. The patient was kept at home for two days following the accident. When Dr. Staub and Dr. Lunsford visited him about twenty-four hours after the attempted suicide, he was found walking about the house. During the first two days, the temperature did not rise above 99 F., and the pulse and the respiratory rates were not increased. Physical signs referable to the heart and lungs were still absent.

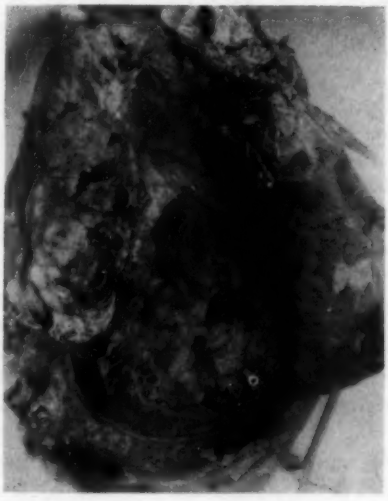
On the third day, the patient was transferred to a private sanatorium. The temperature was now 101 F., the pulse rate 86 and the respiration rate 26 per minute. On the fourth day, appreciable change did not occur. A friction rub was heard for the first time over the precordial region on the fifth day, but other adventitious sounds were not heard. A roentgen-ray examination of the chest failed to reveal the presence of a foreign body. The lack of evidence of a foreign body, together with the continued good condition of the patient, led the attending physicians to doubt that the tube was actually present in the chest. On the sixth day, the temperature was 102.4 F., with a corresponding rise in the pulse rate. Respirations were 22 per minute. On the seventh day, the pulse rate was 118 per minute, and the temperature 103 F. On the eighth day, the temperature rose to 103.4 F., the pulse rate to 122 and the respiration rate to 42 per minute. The fever was at all times continuous and not of the septic type. At 2 a. m. on the day of death and eight days after the accident had occurred, the patient suddenly became cyanotic, with rapid pulse and respirations. This lasted for approximately an hour and was followed by improvement. Twelve hours later, the same symptoms returned, and the patient sank rapidly and died after a period of ten minutes.

Observations at Autopsy.—The body was that of a well developed and well nourished white man 47 years of age. Between the fifth and sixth ribs, 3 cm. to the left of the sternal margin, were three areas, each about 5 mm. in diameter, where the skin had been denuded, which were now covered by dry crusts.

When the chest was opened, a small, ragged wound was found in the intercostal muscles between the fifth and sixth ribs on the left side. A thin layer of dark, clotted blood extended from the fourth to the seventh rib on this side. In the anterior surface of the parietal pericardium near the apex of the heart was a small wound which had been sealed off with fibrin. The inner surface of the pericardium at this point showed a pitlike depression with the base directed outward. The parietal pericardium was everywhere thickened, and infiltrated with blackish, clotted blood. The pericardial sac was obliterated by an exudate, chiefly

fibrinous, but with a considerable admixture of clotted blood over the anterior surface of the heart. The heart was moderately increased in size.

Two centimeters from the apex of the left ventricle anteriorly and slightly to the left of the anterior interventricular branch of the left coronary artery, an aluminum tube projected outward from the surface of the heart for a distance of nearly 2 cm. into the pericardial sac. The visible end of the tube was blunt. A section across the ventricles of the heart showed the tube passing through the anterior wall of the left ventricle just to the left of the interventricular septum, and the base of the anterior papillary muscle of the mitral valve and tearing through the trabeculae carneae and entering the ventricular cavity at the apex. At this point, there was a small, fibrinous thrombus between two trabeculae carneae. The tube then passed through the posterior wall of the ventricle, taking a course slightly to the left from the point of entrance anteriorly, through the pericardial sac and the parietal pericardium, and entered the medial surface of the



Anterior view of the heart with the aluminum pipestem in the position in which it was found at autopsy.

left lung. The tube was found in the position described. For a distance of from 3 to 5 mm. about the hole made by the tube, the myocardium appeared pale and friable. A considerable amount of old and recent hemorrhage was present in the portion of the left lung into which the tube had passed.

The aluminum tube measured 8.9 cm. in length and 4 mm. in diameter. The end which had penetrated the heart was shaped like a trochar and was fairly sharp in spite of the tip having been bent backward over the beveled surface. Toward the sharpened end the tube was coated by an adherent, blackish, gummy substance, apparently nicotine. The lumen of the tube was plugged with clotted blood.

The left pleural cavity was obliterated by fibrous adhesions laterally and at the base and by fibrinous exudate on the surface adjacent to the pericardium. The visceral pleura was markedly thickened and fibrous. In the anterior portion of the lower lobe of the left lung there was a small, encapsulated, caseous area 5 mm. in diameter. A tubercle was also present in one of the hilum lymph nodes.

Tuberculous lesions were not observed in the apexes of the lungs. The right lung was free from changes other than passive hyperemia.

The kidneys and the liver were swollen and pale, but were otherwise negative. The spleen was moderately increased in size and on sectioning appeared hyperplastic. Evidence of a foreign body was not found in the alimentary tract.

Microscopic Examination.—Sections of the left ventricle including the opening made by the tube revealed a quantity of cellular detritus and masses of lime salt about the margins of the wound. The periphery of the opening was composed of a more or less laminated, fibrinous exudate containing only a few leukocytes. At one point the wound extended into the ventricular cavity and the space between the trabeculae carneae was filled with fibrin and broken-down erythrocytes. For a short distance peripheral to the fibrinous exudate, the heart muscle cells had undergone necrosis and only the stroma remained intact. Here there was active proliferation of the supportive structure cells with the formation of numerous new blood vessels. Wandering cells laden with yellowish, granular pigment were present in this zone. Elsewhere the myocardial muscle showed slight Zenker's necrosis and brown atrophy. Evidences of infection about the wound were almost wholly lacking.

The blood vessels in the subepicardial fat were moderately engorged, and in the visceral pericardium an active fibroblastic and angioblastic proliferation was occurring. Here also were numerous old hemorrhages with disintegration of the cells and formation of blood pigment. Over the surface was a fibrinous exudate.

Sections of the parietal pericardium showed a picture similar to that described for the visceral layer. The fat outside the sac was infiltrated with blood, which was breaking down to form hematoidin pigment.

Microscopic sections of the left lung disclosed an old hemorrhage in the medial portion. In the thickened pleura were moderate numbers of miliary and conglomerate tubercles, but only one tubercle occurred in the lung itself. All appeared to be active. One of the hilum lymph nodes contained a caseous tubercle with several miliary tubercles about its margin. The only noteworthy change observed in sections from the right lung was passive hyperemia.

Sections of the liver and the kidneys revealed parenchymatous degeneration and passive hyperemia. In the spleen was evidence of increased destruction of blood and hyperplasia of the lymphoid follicles.

Pathologic Diagnosis.—Penetration of the left ventricle of the heart by a foreign body (an aluminum tube from a pipestem); necrosis and early fibrosis of the myocardium and fibrinous thrombosis of the left ventricle; old hemato-pericardium; subacute fibrinous pericarditis with obliteration of the pericardial sac; old intrapulmonary hemorrhage in the left lung due to partial penetration by foreign body; old hemorrhage into the anterior mediastinum; perforating wound of the anterior thoracic wall; general acute passive hyperemia; cloudy swelling of parenchymatous organs; active tuberculous pleuritis, obliterating the left pleural cavity, and active caseous tuberculosis of a hilum lymph node.

SUMMARY

The outstanding clinical facts were the striking absence of physical signs and symptoms in the presence of what proved at autopsy to be a severe cardiac injury. Only the wounds in the skin of the chest, the friction rub over the precordial area and the increasing temperature and accelerating pulse and respiration rates pointed toward an intra-

thoracic pathologic process. Not until late did symptoms of cardiac failure appear. Because of the indefiniteness of symptoms and the inability to locate the body with the roentgen ray, it was deemed inadvisable to subject the patient to an exploratory operation. As aluminum is penetrated by all except the shortest of the roentgen rays, the difficulty of locating an aluminum object by this means is obvious. The clinical course would indicate that death resulted from cardiac failure, although the possibility of an infection of the blood stream was not excluded.

The position of the tube would suggest that it had been forced entirely through the organ by the patient, since it is hardly possible for it to have migrated to the position in which it was found at autopsy. The tube was held firmly in the heart by a layer of fibrin and must have moved back and forth with each contraction of the organ. The slight hematopericardium may be explained by the fact that the tube barely entered the ventricular cavity, and that it was quickly surrounded by fibrin. The distal end projected into the medial surface of the left lung and the lumen was plugged with clotted blood. With the passage through the heart of a contaminated object such as this must have been, one would expect some degree of infection of the injured structures; but the microscopic study does not confirm such a supposition. The gross and microscopic study of the various organs supports the clinical evidence of a relatively acute circulatory failure.

THE LESIONS IN ANIMALS INOCULATED WITH STREPTOCOCCUS CARDIO-ARTHRITIDIS *

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The production of experimental lesions of rheumatic fever has been attempted by a number of workers with different streptococci, including *S. viridans, S. hemolyticus* and *S. nonhemolyticus*.[1] In most instances, these investigators considered their results more or less similar to the anatomic changes described by Aschoff and others in human tissues, and some described their experimental lesions as typical. There is, however, a difference of opinion concerning the specific character of any of these lesions. Thalheimer and Rothschild,[1] after reviewing the subject, were convinced that none of them was typical of rheumatic fever.

The isolation by Small,[2] in 1926, of a nonhemolytic streptococcus (*Streptococcus cardio-arthritidis*) in cultures from the blood of two patients having typical cases of rheumatic fever, and the subsequent collection by himself [3] and Kreidler [4] of 107 strains of this streptococcus, stimulated interest in the subject. The important feature of their work was that all of these strains were biologically similar; that is, the strains of *Streptococcus cardio-arthritidis* constituted a specific serologic group. This is the first such definite group of streptococci to be associated with rheumatic fever—a consideration which prompted the present study.

MATERIAL AND TECHNIC

The anatomic lesions here described were found in seven of eight rabbits, and in two horses inoculated with *S. cardio-arthritidis*. Dr. John Reichel of the H. K. Mulford Company's Laboratories provided us with an opportunity to study the tissues of the horses. The rabbits were given viable twenty-four hour cultures of *S. cardio-arthritidis* suspended in saline solution. The number of streptococci for each injection was determined by standardizing the suspensions

* From the Laboratory of the Philadelphia General Hospital.

1. Poynton and Payne: Lancet **2**:861, 1900; **1**:1260 and 1524, 1901; Tr. Path. Soc. London **52**:248, 1901. Bracht and Wachter: Deutsches. Arch. f. klin. Med. **96**:493, 1909. Coombs and Miller: Lancet **2**:1209, 1912. Rosenow: J. Infect. Dis. **11**:210, 1912. Jackson: Ibid. **11**:243, 1912. Rosenow and Coombs: Lancet **2**:1692, 1913. Thalheimer and Rothschild: J. Exper. Med. **19**:417 and 429, 1914. Clawson: J. Infect. Dis. **36**:444 (May) 1925.

2. Small, J. C.: Am. J. Med. Sc. **173**:101, 1927.

3. Small, J. C.: Am. J. M. Sc. **175**:638, 1928.

4. Kreidler, W. A.: J. Infect. Dis., to be published.

as in the preparation of vaccines. The size and the number of doses were determined by the clinical behavior of each animal. In all but one instance, Small's original strain R1 was used. In the one instance, strain R9, also recovered in blood culture from a typical case of rheumatic fever, was used. Two animals, the protocols of which are not included, died within a few hours after the first injection.

S. cardio-arthritidis was recovered in culture from the lesion in the heart of rabbit 1,[5] re-injected into rabbits 4, 5 and 6, and again cultured from the lesions of rabbit 4. The lesions of rabbits 9 and 10 were produced with this culture after its passage through rabbits 1 and 4. This fulfilled the requirements of Koch's postulates, so far as the passage of the virus through animals was concerned.

Rabbit 1.—On July 12, 1927, the weight of the animal was 2,024 Gm. The hemoglobin content of the blood was 55 per cent, and the erythrocytes numbered 4,460,000. Figure 1 presents the details of the clinical course. Symptoms other

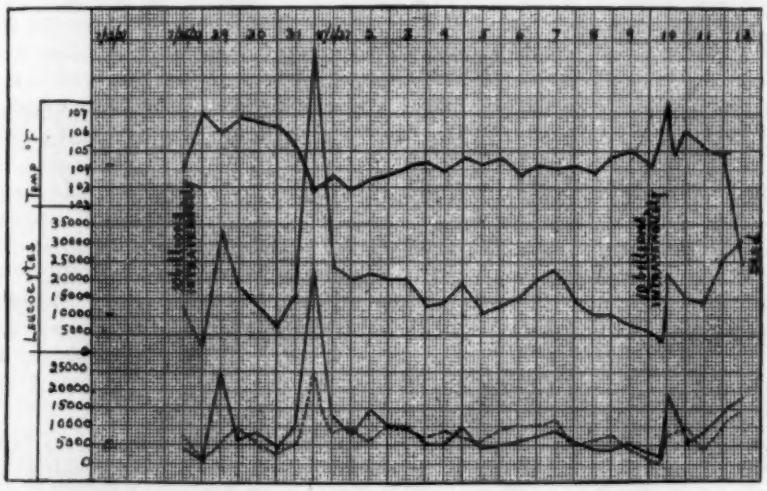

Fig. 1 (rabbit 1).—A chart of the clinical course of the reaction of rabbit 1 after an inoculation with Streptococcus cardio-arthritidis, strain R1. In the two curves in the lower part of the chart, the solid line indicates polymorphonuclears and the broken line lymphocytes.

than those ordinarily associated with febrile reactions were not observed; a transient rhinitis followed each injection. There was not an appreciable loss in hemoglobin. The weight, though fluctuating somewhat, was fairly well maintained.

At autopsy, which was done thirty hours after the death of the rabbit, the heart was found flabby, the chambers dilated, and the valves normal, with the exception of the mitral. Around the margins of this valve were large, white, somewhat friable vegetations projecting into the ventricle and extending a little way on the chordae tendineae. *S. cardio-arthritidis* was recovered from these vegetations, together with a gram-negative bacillus of the colon type. Other gross

5. R1A was used to designate this strain after a passage through one animal; R1B, after a passage through two animals.

lesions, except congestion and cloudy swelling of the viscera, and an abscess in the ear at the point of the injection, were absent. An elbow joint, boiled and cleaned, did not present gross abnormalities.

As may be seen in figure 1, immediately following the injection the temperature rose for a few hours, while the number of leukocytes decreased. This was followed by a fall in temperature with a synchronous increase in the number of the white blood cells. The leukocytic reaction was chiefly polymorphonuclear, though the lymphocytes participated somewhat more tardily. This phenomenon was complete in from twelve to eighteen hours. It was a reaction that occurred rather characteristically in each of the rabbits after every injection. It was not considered in any sense a specific phenomenon, but as one which might result from the intravenous injection of any bacterium in large amounts.

The marked leukocytosis and the fall in temperature developing spontaneously in this animal on the third day after the first injection were interesting. The phenomena probably indicated the date of actual infection following a period of incubation.

The method of study followed in reference to this animal was practiced with the others, although in some of the later cases it was found sufficient to make observations less frequently. The behavior of the large mononuclear cells and of the eosinophils was not recorded, variations in these seeming to be insignificant.

Rabbit 2.—On July 12, 1927, the weight of the animal was 1,637 Gm. The hemoglobin content of the blood was 52 per cent; the erythrocytes numbered 3,920,000; the leukocytes, 6,500, of which the polymorphonuclears formed 54 per cent, and the lymphocytes, 41 per cent. The temperature was 103.2 F. Between July 14 and August 16, the animal was given five intravenous injections of *S. cardio-arthritidis,* strain R9; four of 10,000,000,000 and one of 7,500,000,000 organisms. A purulent rhinitis appeared after each of these injections. On August 27, the animal developed diarrhea and died.

The autopsy, performed about twelve hours after the animal's death, did not disclose any gross lesions. Bacteriologic studies were not made.

Rabbit 4.—On August 29, 1927, the weight of the rabbit was 2,445 Gm. The blood showed a hemoglobin content of 55 per cent; the erythrocytes numbered 4,420,000; the leukocytes, 6,900, of which the polymorphonuclears formed 46 per cent, and the lymphocytes, 49 per cent. The temperature was 103.5 F. The animal was given, intravenously, 5,000,000,000 of *S. cardio-arthritidis,* strain R1A, which had been recovered from rabbit 1. There occurred an immediate reaction as noted above. On August 31, the temperature was 104 F. The leukocytes numbered 31,200, of which the polymorphonuclears formed 70 per cent. A purulent rhinitis had developed. On September 3, the rabbit was apparently well. On September 7, 5,000,000,000 of *S. cardio-arthritidis* (R1A) were given intravenously. On September 8, the right knee of the rabbit was swollen and tender. On September 9, fluid aspirated from the right knee contained pus, but did not reveal bacteria on smears. The cultures produced an occasional colony of *Staphylococcus aureus.* On September 13, a slight leukocytosis had been present since September 4. Five billions of *S. cardio-arthritidis* R1A were injected intravenously. Malaise and rhinitis developed. Cultures of the nasal discharge were negative for *S. cardio-arthritidis.* On September 15, the animal was apparently normal, except for a slight rhinitis. On September 23, 5,000,000,000 of *S. cardio-arthritidis* R1A were injected intravenously. Later in the day, the right knee was again swollen and the rhinitis was more severe. Cultures of this discharge were negative for *S. cardio-arthritidis.* On September 24, diarrhea developed. On September 28, the temperature was 104.1 F. The leukocytes numbered 13,400. Five billions of *S. cardio-arthritidis*

R1A were injected intravenously. On September 30, the temperature was 105 F. The leukocytic count was 7,600. On October 2, the hemoglobin content of the blood was 51 per cent; the erythrocytes numbered 4,000,000, and the leukocytes 15,000. The temperature was 106.6 F. The weight was 2,275 Gm. The animal appeared ill, and was markedly fatiguable and dyspneic. There appeared to be exostoses about the right knee. On October 4, 2,500,000,000 of *S. cardio-arthritidis* were injected intravenously. On October 5, the weight was 1,085 Gm. The temperature was 101.3 F. The leukocytic count was 32,400. The animal was put to death.

Autopsy, which was done at once, showed the heart flabby and the valves normal, with the exception of the mitral. Almost all the auricular surfaces of this valve were covered with large, white, rather friable vegetations, which narrowed the orifice. These extended to the attachments of the papillary muscle. *S. cardio-arthritidis* was recovered in pure culture from the heart blood (R1B), and it was recovered from the mitral vegetations in association with a gram-negative bacillus of the colon type. One infarct was found in the spleen and in each kidney, and an abscess in the rectum, probably from the repeated insertion of the thermometer. The right knee joint, boiled and cleaned, did not present any gross lesion.

Rabbit 5.—On Aug. 29, 1927, the weight of the animal was 2,527 Gm. The temperature was 104 F. The hemoglobin content of the blood was 86 per cent; the erythrocytes numbered 6,380,000; the leukocytes, 12,300, of which the polymorphonuclears formed 31 per cent and the lymphocytes, 62 per cent. The animal was given intravenously, 2,500,000,000 of *S. cardio-arthritidis* R1A, which had been recovered from rabbit 1. On August 31, purulent rhinitis developed. On September 7, the weight was 2,115 Gm. The temperature was 102.8 F. The leukocytes numbered 24,200, of which the polymorphonuclears formed 47 per cent. Two billions of *S. cardio-arthritidis* R1A were injected intravenously. On September 8, a soft, fluctuating mass was noted in the region of the right parotid and on the lateral aspect of the right knee. The left parotid area seemed swollen. On September 9, the swellings of the right parotic region and of the knee were aspirated, yielding a clear, straw-colored fluid, which was without pus or bacteria, on smear. The cultures showed some staphylococci. On September 13, the rabbit had had a moderate leukocytosis since the first injection, without fever. Two and one-half billions of *S. cardio-arthritidis* R1A were injected intravenously. Later in the day the rabbit appeared ill, and had rhinitis. On September 23, the animal was apparently normal with the exception of a swelling in the region of the right knee. Two and one-half billions of *S. cardio-arthritidis* R1A were injected intravenously. Later, the knee was more swollen. On September 28, 2,500,000,000 of *S. cardio-arthritidis* R1A were injected intravenously. On October 4, the rabbit appeared normal except for swellings in the right parotid region and on the outer aspect of the right knee. It was given 5,000,000,000 of *S. cardio-arthritidis* R1A intravenously. On October 10, the weight of the rabbit was 2,375 Gm. The temperature was 103.8 F. The hemoglobin content of the blood was 70 per cent; the erythrocytes numbered 5,750,000, and the leukocytes, 12,700. On October 28, the rabbit was apparently well, except for the articular swellings. It was put to death.

At autopsy, which was performed immediately, sacs containing colorless fluid were dissected out from the region of the right knee and the right temporomandibular articulation. In this fluid were strands of gelatinous material, on which were small, oval, white bodies. Other gross lesions did not appear. The culture from the heart blood was negative for *S. cardio-arthritidis.*

Rabbit 6.—On Oct. 6, 1927, the weight of the animal was 2,625 Gm. The temperature was 103.2 F. The hemoglobin content of the blood was 91 per cent; the erythrocytes numbered 6,960,000 and the leukocytes, 9,950. On October 14, 5,000,000,000 of *S. cardio-arthritidis* R1A from rabbit 1 were injected intravenously. On October 21, the rabbit had had a slight continuing leukocytosis, with normal temperature. The weight of the animal was 2,635 Gm. The temperature was 103.3 F. The leukocytic count was 25,800. On October 27, the blood culture was sterile. On November 8, the right eye was infected. On November 10, the weight was 2,400 Gm. The temperature was 102.5 F. The hemoglobin content of the blood was 72 per cent; the erythrocytes numbered 5,010,000. The leukocytes numbered 19,200, of which the polymorphonuclears formed 71 per cent. Panophthalmitis of the right eye developed. On November 14, the rabbit's condition was unchanged. Five billions of *S. cardio-arthritidis* R1A were injected intravenously. On November 22, 5,000,000,000 of *S. cardio-arthritidis* R1A were injected intravenously and 11,000,000,000 subcutaneously. On November 23, the weight was 2,215 Gm. The temperature was 102 F. The leukocytes numbered 44,000. The rabbit was put to death.

The autopsy, which was done at once, revealed a small, white, firm nodule, about 1 mm. in diameter on the mural endocardium of the left ventricle. Other lesions, except the panophthalmitis, were not found. Bacteriologic studies were not made.

Rabbit 8.—On Dec. 1, 1927, the weight of the animal, a young one, was 1,665 Gm. The temperature was 102.6 F. The hemoglobin content of the blood was 85 per cent. The animal was given, intravenously, 4,500,000,000 of *S. cardio-arthritidis* R1B recovered from rabbit 4, and as much again, subcutaneously. On December 2, the animal was placed in the refrigerator at —10 C. for about fifteen minutes, then given 10,000,000,000 of *S. cardio-arthritidis* R1B intravenously. On Jan. 19, 1928, the rabbit did not appear ill. It was put to death.

An autopsy was performed at once. Gross and microscopic examinations both failed to disclose any lesions. The culture of the heart's blood was sterile.

Rabbit 9.—The weight of the animal, like the former, a young one, was 1,220 Gm. The temperature was 103.3 F. The hemoglobin content of the blood was 70 per cent. The erythrocytes numbered 4,970,000; the leukocytes, 6,600, of which the polymorphonuclears formed 52 per cent, and the lymphocytes, 36 per cent. The rabbit was given, intravenously, 3,400,000,000 of *S. cardio-arthritidis* R1B from rabbit 4, and as much again subcutaneously. On Dec. 2, 1927, the animal was placed in the refrigerator at —10 C. for about fifteen minutes, and then was given 10,000,000,000 of the same culture R1B, intravenously. Later in the day a bloody diarrhea developed. On December 6, the animal had apparently not been infected. The temperature was 101.4 F. The weight was 875 Gm. On December 9, the animal was found dead.

Autopsy was performed several hours after its death. On the tricuspid valve were several small, warty masses, the largest of which was about 3.5 mm. in diameter, the smallest about 0.5 mm. The largest was attached to a papillary muscle as well as to the valve. Cultures from the center of this vegetation showed *Bacillus coli* and a diphtheroid bacillus, but no *S. cardio-arthritidis*. These were considered contaminants. Other gross lesions did not appear. A knee joint was dissected and found to be normal.

Rabbit 10.—A young animal, weighing 1,630 Gm. was used. The temperature was 103.8 F. The hemoglobin content of the blood was 72 per cent; the erythrocytes numbered 5,310,000; the leukocytes, 13,100, of which polymorphonuclears

formed 53 per cent and lymphocytes, 43 per cent. The animal was given 4,500,000,000 of *S. cardio-arthritidis* R1B from rabbit 4, intravenously, and as much again subcutaneously. On Dec, 2, 1927, the animal was placed in the refrigerator at — 10 C. for about fifteen minutes, and then given 10,000,000,000 of the same strain intravenously. On December 9, the animal appeared well. The weight was 1,490 Gm. The temperature was 103.7 F. On Jan. 19, 1928, the animal was killed.

Autopsy, done at once, disclosed, on and near the tricuspid valve, four white, smooth, firm, globoid masses, one of which attached the valve to the wall of the ventricle. The largest of these, about 2 mm. in diameter, was on a papillary muscle. Those on the valve were less than 1 mm. in size. Cultures from the center of the larger vegetation showed diphtherioid bacilli, and a few staphylococci. These were considered contaminants. A culture of the heart blood was sterile. Other gross lesions were absent. Both knee joints were dissected and found to be normal.

Tissues were fixed in 4 per cent solution of formaldehyde, physiologic sodium chloride and stained with hematoxylin and eosin.

MICROSCOPIC STUDIES

The heart of each of the rabbits with disease showed a moderate degree of cloudy swelling and slight fatty degeneration. Many muscle cells, however, even near the areas of inflammation, retained their cross-striations and appeared to be normal. In the animals which had received intravenous injections within a day or two of death, small areas of degenerated muscle fibers were found, in which polymorphonuclear cells had collected. These areas were such as would be expected to result from the intravenous injection of any bacterium, and are not to be considered as having any relation, or similarity to lesions of rheumatic fever.

In six of the eight rabbits there appeared, more or less well developed, but scattered, small collections of endothelioid cells about capillaries and small arteries. From five to twenty cells constituted such a focus. In many instances, but not all, the endothelium of the capillaries, or arteries, was swollen and proliferative, or desquamated, and the muscle of the arteries was also swollen. The arterial lesions were well developed in four of the animals, even in vessels of fair size, sometimes without perivascular cellular reaction. In rabbit 4, the adventitia and media of such an artery in the epicardium contained a definite collection of endothelioid cells, forming an Aschoff-like nodule except for the absence of giant cells.

In the left ventricle of rabbit 1, a rather large area near the endocardium was composed of small collections of muscle fibers (from six to eight) which had undergone a degeneration characterized by hyalinization and fragmentation. In about half of these, there had been deposited a light blue substance (hematoxylin and eosin stain), evidently calcium. About these areas were many large cells of irregular shape, some oval, others almost square, with an irregular amount of cytoplasm

staining faintly pink, or faintly blue. The nuclei of these cells were relatively large and conspicuous, basophilic, but very faintly so for nuclei. In them the chromatin was scattered as dots, or in a small pyknotic mass, or distributed as a line in the long axis of the nucleus. The largest of these were the most basophilic, and such types were grouped in areas to form giant cells with six to eight nuclei. About and in these areas was a definite proliferation of connective tissue cells. At some distance from this area of acute calcification, a few interstitial

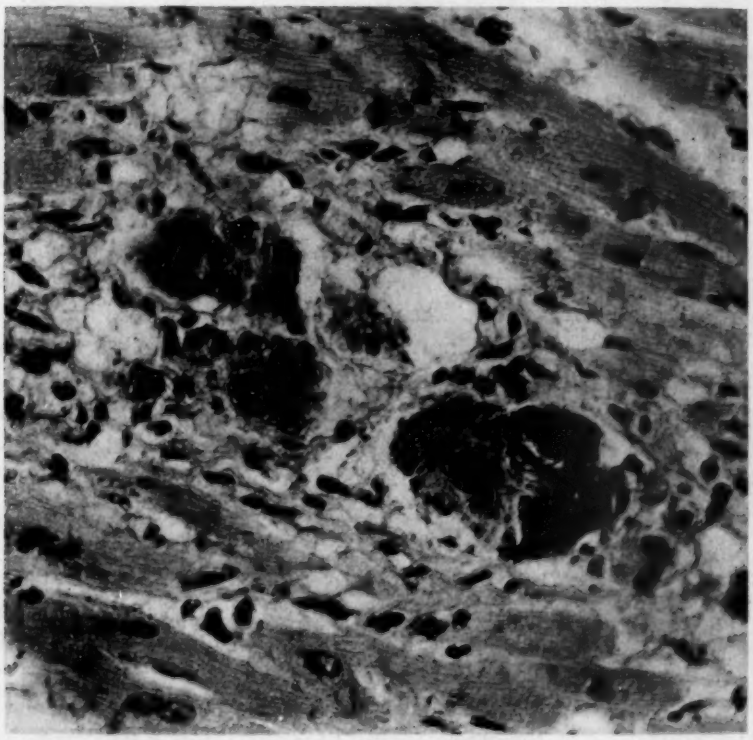

Fig. 2 (rabbit 1).—This shows an Aschoff nodule in the wall of the left ventricle, composed of endothelioid cells forming giant cells, about which are a few lymphocytes and fibroblasts. Calcium is not present in this area; × 552.

lesions were found composed of endothelioid cells, with some lymphocytes, connective tissue nuclei, and a rare polymorphonuclear cell. In some of these, giant cells were well developed, but vascular channels were not seen. One such giant cell contained twenty-three nuclei. Near the large area, however, several instances of arteritis were found. Our Unna-Pappenheim stains were generally not successful, but in the lesions just described sufficiently good differentiation was obtained to show the endothelioid cells and giant cells in deep red colors. Giant cells appeared

in two other animals (9 and 10), but in less characteristic fashion, in or near verrucose lesions. In rabbit 1, there appeared, also, a rather large dendritic area, apparently corresponding to the distribution of an artery, in which muscle cells were atrophic and missing. This was unaccompanied by cellular reaction, or infiltration. Possibly this lesion was due to arteritis. We have seen such areas in human rheumatic hearts.

At the auriculoventricular junction in the hearts of two rabbits (4 and 9) were small areas of edema and congestion, in which appeared lymphocytes, a few polymorphonuclear cells and many endothelioid

Fig. 3 (horse 1).—This shows a perivascular interstitial lesion in the wall of the left ventricle. The endothelioid cells have not formed giant cells. This lesion appeared in eight of the animals, and is described as that type which has not attained the characteristics of a fully developed nodule; × 460.

cells. Some of the blood vessels were damaged. These patches were typical in every respect of epicarditis.

Vegetative endocarditis was found on the valves of two animals (1 and 4), the vegetations consisting of fibrin, pus cells and clumps of gram-positive cocci. The larger masses were on the valve surface, but smaller and presumably less developed foci were distinctly within the valve substance. The verrucose lesions in rabbits 6, 9 and 10 on the tricuspid valves and the mural endocardium of the ventricles appeared to be covered with swollen and eroded endothelium continuous with that

of the nearby structures. The larger masses had central foci of pus cells and cocci, about which were zones of typical endothelioid cells and young fibroblasts. In one of these vegetations, nodules with giant cells had formed, and a single giant cell appeared in the myocardium just under a mural lesion. The smaller lesions had a matrix of basophilic, granular, necrotic material in which were numerous endothelioid cells, a few lymphocytes, but not any pus cells or clumps of bacteria.

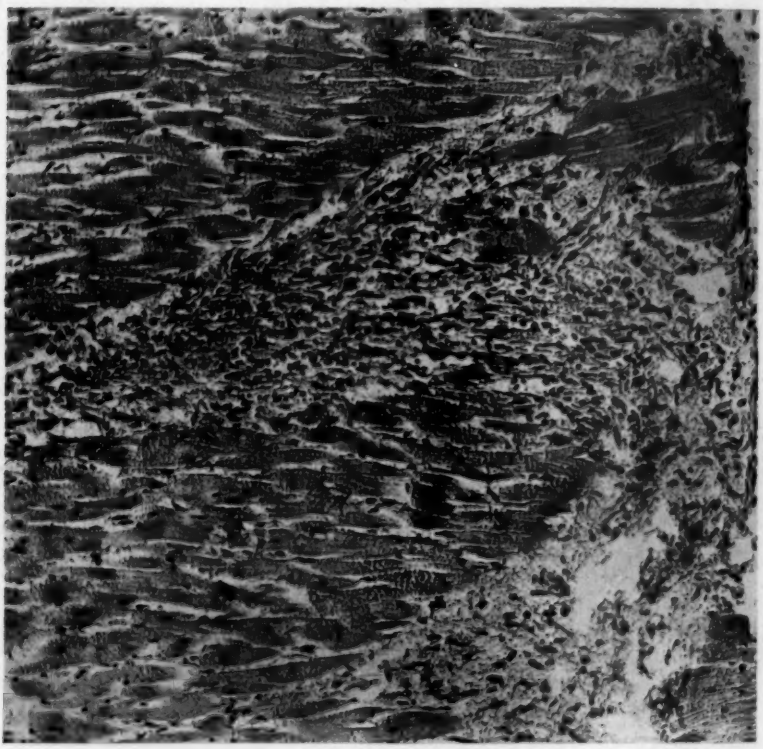

Fig. 4 (horse 1).—This shows an area of active proliferation of endothelioid cells in the interstitial tissue of the myocardium. Giant cells are not present. The blood vessel cannot be recognized; × 138.

In three rabbits, small aortic lesions were found. In one of these, a few large, pale cells were seen, and there appeared to be some destruction of elastic fibers. This could not be stained by the method described by Pappenheim and von Glahn,[6] as recuts did not contain the little area. In two other instances, the lesions were similar to those described by Pappenheim and von Glahn as areas deficient in muscle nuclei, but without damage to elastic tissue. These lesions were not striking, but

6. Pappenheim and von Glahn: J. Med. Research **44**:489, 1923-1924.

are worthy of mention for purposes of correlation with those described by these writers in human aortas.

In rabbit 1, sections of the diaphragm and of a muscle near a knee joint showed an interesting myositis. This appeared as a definite necrosis of numerous isolated muscle cells, the neighboring cells being unaffected. In many instances, numerous nuclei were proliferating in the midst of the degenerated sarcoplasm, forming giant cells. As the

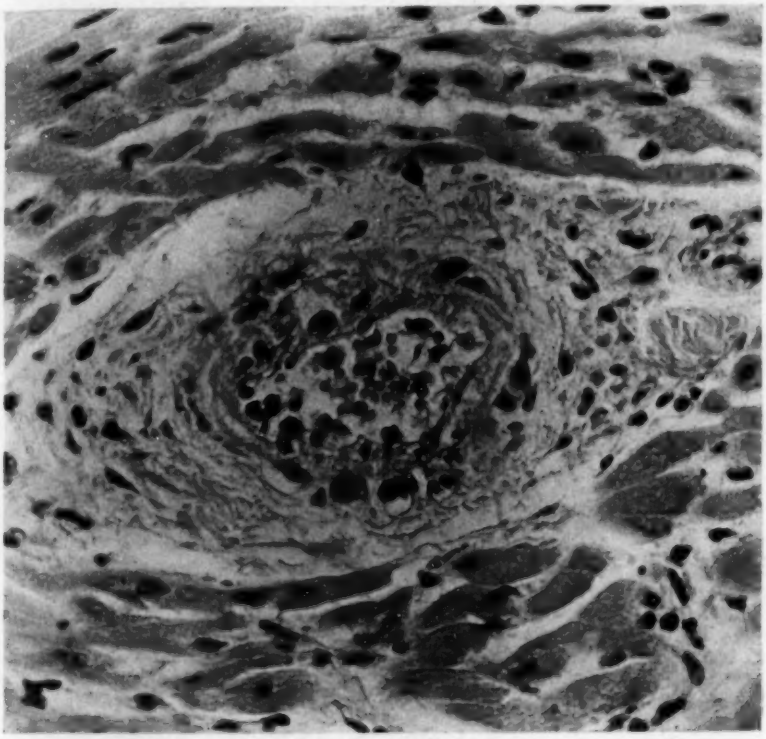

Fig. 5 (rabbit 1).—The type of inflammation of small blood vessels illustrated in this artery, was seen in six of the animals; × 552.

whole process appeared so well confined to the area of the damaged cells, the proliferating nuclei were considered to arise from the sarcolemma, and not from the surrounding tissue. Sections from the periarticular tissues of two other animals (4 and 5) showed little collections of endothelial cells, lymphocytes and, in rabbit 4, of pus cells, where muscle and aponeurosis joined. In some instances, a damaged capillary was seen in the midst of the nodule. In the purulent areas of rabbit 4, some bacilli and cocci could be seen, and also in the bone marrow, but in the other animal, the inflammatory nodules were rather

typical of those described by Swift [7] and others. The bursitis of rabbit 5 was a subacute suppurative inflammation, but nodules were not found in its wall. The white bodies described grossly were "joint mice." In these were several small collections of cloudy, bluish endothelioid cells suggestive of nodules.

Horse 1.—The animal was given subcutaneous injections of living *S. cardioarthritidis* mixed with citrated blood over a period of eight and one-half months. The maximum dose was 250,000,000,000 organisms. Clinical symptoms failed to develop, with the exception of a rise in temperature to 103 F. and 105 F for a

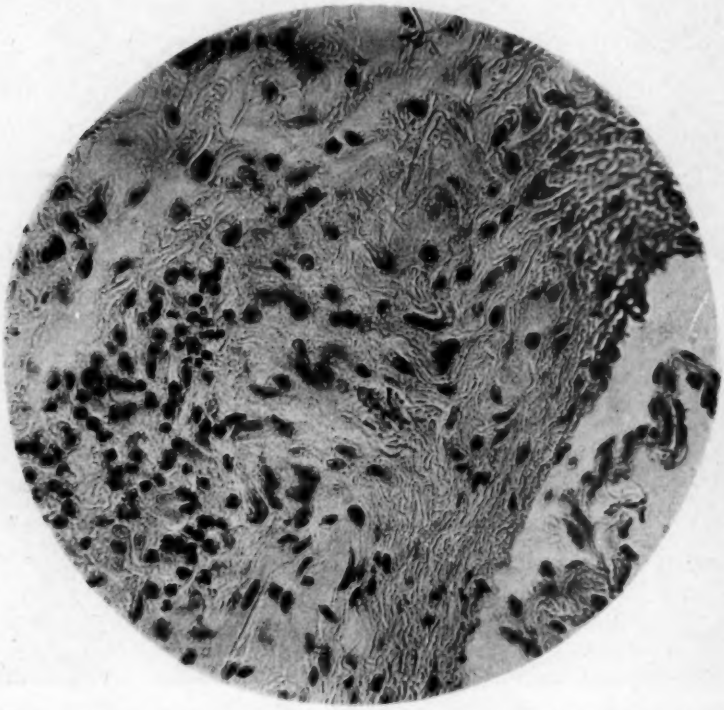

Fig. 6 (rabbit 4).—Inflammation is shown in the wall of an artery in an area of pericarditis. The proliferating endothelioid cells occupy the adventitia and most of the media of this vessel; × 322.

week before death. At autopsy, the heart was found to be enlarged and hemorrhagic, and the pericardium inflamed. The mitral valve was somewhat thickened along its edges, apparently by edema. There was inflammation also of the peritoneum; the kidneys, lungs and spleen were congested, and the liver was enlarged.

Horse 2.—This animal was treated as was horse 1, and for the same period of time. The only clinical symptom was a rise of temperature to 102 F. and 103 F. for two days before death. Autopsy showed a dilated heart with a hemorrhagic pericardium. The lungs were congested and the pleura was thickened. The liver and the spleen were enlarged. Over the surface of the intestines were petechiae.

7. Swift: Am. J. Med. Sc. **170**:631, 1925.

Microscopic sections of the pericardium from each of the horses showed congestion, edema and an infiltration with lymphocytes and pus cells. There was a proliferation of endothelioid cells, particularly about small blood vessels. In the myocardium were small perivascular lesions, similar to those in the epicardium, and to those in most of the rabbits. In addition, especially in horse 1, there were areas of fair size between muscle bundles, where marked proliferation of endothelioid cells and young connective tissue cells had occurred. The background of these

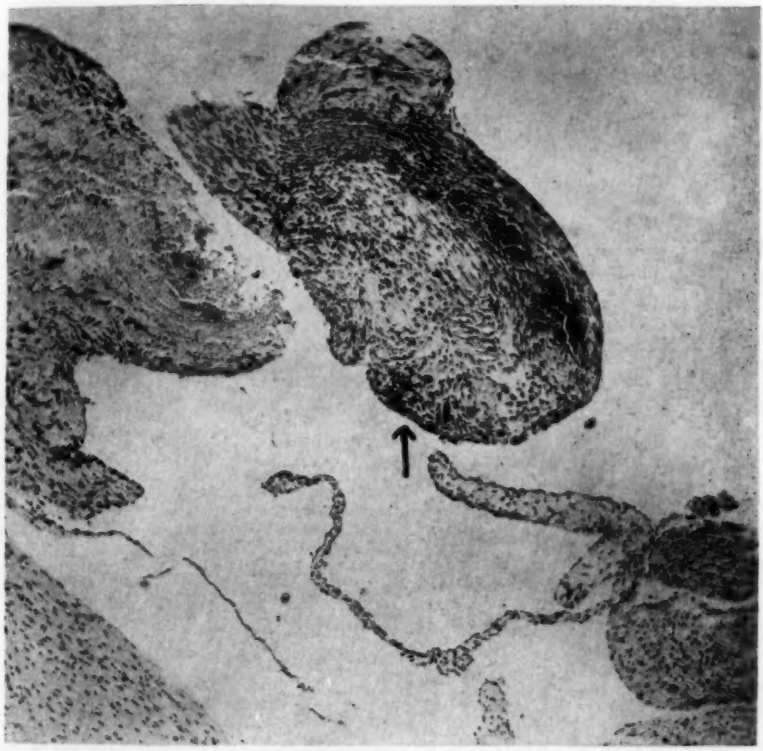

Fig. 7 (rabbit 9).—This is a cross-section of one of the verrucose lesions on the tricuspid valve. The dark dots are clumps of cocci, about which are a few pus cells. The greater part of the mass is composed of endothelioid cells and fibroblasts. A small collection of fibrin is attached at one point; × 80.

was a granular material staining faintly pink. A few lymphocytes and polymorphonuclear cells were also present. An association of these areas with blood vessels was not evident. Elsewhere several instances of well developed arteritis were seen. Within the tissues of the mitral valves, there were dilated and apparently newly formed blood capillaries. About some of these were collections of endothelioid cells forming fairly typical nodules, except for the absence of giant cells. The valves contained also areas of fatty and hyaline degeneration and of scar tissue.

In the lungs of all animals, both horses and rabbits, there was thickening of the alveolar walls, especially at the nodal points. This thickening was due to congestion, edema, infiltration with lymphocytes and a few polymorphonuclear cells, and to swelling and apparent proliferation of endothelial cells. This anatomic change, on superficial study, had the appearance of a partial atelectasis. Closer study showed the air spaces to be reduced in area, not by collapse, but by the interstitial inflammation.

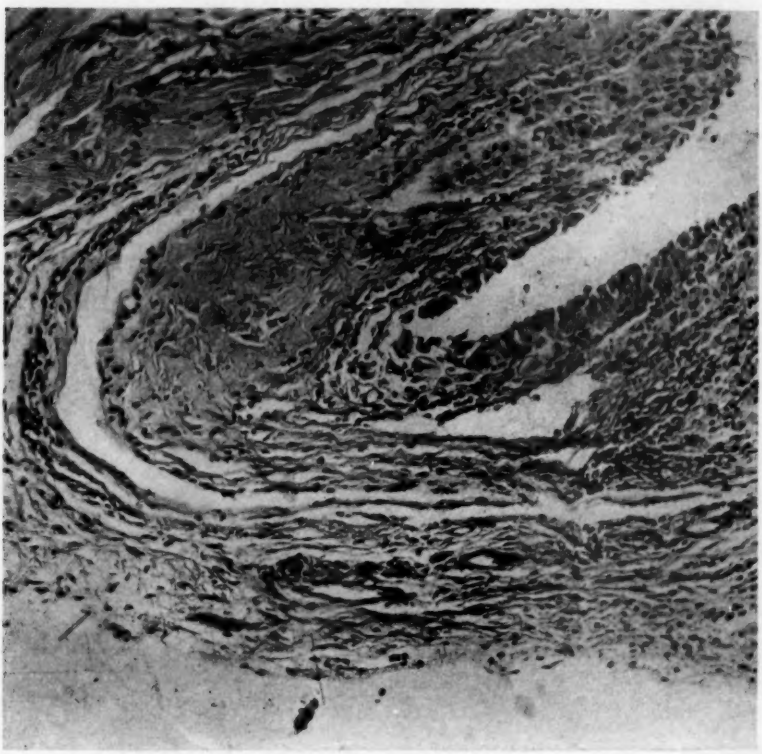

Fig. 8 (rabbit 8).—This illustrates the type of inflammation in the bursae and the periarticular tissues of the knee joint; × 138.

Summarizing the lesions, one finds in reference to the rabbits, that epicarditis appeared in two animals; nonpurulent focal myocarditis in six; mural endocarditis in three; valvular endocarditis in four; arteritis in four; myositis (including periarthritis) in three; bursitis in one; glomerular nephritis in one; infarcts of spleen and kidney in one; aortitis in three, and pneumonitis in eight; and that epicarditis, focal myocarditis, valvular endocarditis, arteritis, and pneumonitis appeared in both horses

COMMENT

Undoubtedly there is difficulty, at times, in determining that a given lesion is typically that of rheumatic fever. This difficulty is experienced even in the study of human tissues, of which only a percentage show characteristic Aschoff nodules. There seems to be agreement, however, that these nodules, when demonstrable, do constitute conclusive evidence of this disease. Evidently, the development of the nodules is influenced by certain variables, of which the duration of infection is probably the most important, although the intensity of the inflammatory process and the individual variations in resistance of the host possibly have an influence. This difficulty is naturally greatest in experiments on animals. Coombs,[8] commenting on this point, says, "The differences between human and experimental lesions are due to the fact that we do not know yet how to reproduce accurately in animal experiments the actual steps by which the organism gains access to the cardiac tissue of the human subject." We have been cognizant of these considerations in attempting to interpret our results.

In our rabbit 1, the interstitial myocardial lesions with giant cells were indistinguishable from typical Aschoff nodules. The myocardial lesions found in other animals were similar to those occurring in rheumatic fever in man at the stage at which the characteristics of fully developed nodules have not been attained. This similarity lies in the focal nature of the lesions, the predominance of proliferative over destructive processes, the endothelioid character of the cellular reaction, and the frequency and the type of the arterial lesions. The vegetative endocarditis in rabbits 1 and 4 may possibly have been influenced by the intravenous injections of streptococci given shortly before death. This is the more worthy of consideration in the light of the fact that the endocarditis in rabbits 6, 9 and 10, in which the last injections were given some days before death, were verrucose. The latter lesions, although active inflammatory processes, had every evidence of taking their origin from the interior of the valves, a characteristic of rheumatic fever emphasized by Coombs,[8] Swift [7] and others. It seems permissible to speculate on the possibility of their hyalinizing and becoming true verrucae in time. The valve changes in the two horses were in some respects similar to those described by Wadsworth [9] in pneumococcal endocarditis in these animals. The appearance, however, of rather characteristic nodules in the animals inoculated with *S. cardio-arthritidis*, and the absence of purulent processes, such as he found, constituted a fundamental difference.

8. Coombs: Rheumatic Heart Disease, Bristol, John Wright & Sons, Ltd., 1924.

9. Wadsworth: J. Med. Research **39**:279, 1918-1919.

The periarticular lesions occurred in rather a small percentage of our rabbits (three of eight). These were, however, especially in rabbits 1 and 5, very like those found in the joint structures of human beings.

In criticism of our results we would mention the paucity of giant cells, which were observed in three animals but in only one in characteristic fashion; the absence of perfectly typical verrucae, and the fact that our lesions were generally less intense, and less widespread than those to be seen in many cases of rheumatic fever.

We have not emphasized the lesion of the lung, although this was the most constant of our observations. We have abundant proof in excellent sections of tissue that this lesion does occur often in rheumatic fever in man. We have, however, found it in persons dying of endocarditis due to *Streptococcus viridans,* in whom a rheumatic element was not apparent; and recently in rabbits inoculated intravenously with *Bacillus influenzae.* We therefore do not consider this peculiar to rheumatic fever, and consequently hold it of little value in this study.

Our material is hardly suitable for the study of the origin of the endothelioid cells of rheumatic fever. In these animals, however, as well as in human material collected for comparison, a number of microscopic fields suggest that these peculiar cells might arise from the muscle cells of small arteries. So far as we are aware, this origin has not been considered before.

In conclusion, this much may be safely said, that *Streptococcus cardio-arthritidis* produced in rabbits and horses anatomic changes strongly resembling those of rheumatic fever, and certainly as nearly characteristic of this condition as were any experimental lesions previously reported as produced by streptococci.

SUMMARY

In rabbits and horses inoculated with *Streptococcus cardio-arthritidis,* lesions were identified which resembled those of rheumatic fever. These lesions are described.

THE HISTOGENESIS OF ESSENTIAL LIPOID HISTIOCYTOSIS (NIEMANN-PICK'S DISEASE)*

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CHICAGO

The opportunity recently afforded me to study some of the material of the new case of essential lipoid histiocytosis reported by Abt and Bloom [1] stimulated me to reexamine the tissues of the three cases that I reported several years ago.[2]

Exclusive of some of the neoplasms, few diseases show as widespread anatomic changes as those which are characteristic of this disease. It affects practically all of the organs and tissues of the body and, in so doing, presents many problems of morphologic and physiologic interest.

Fifteen cases of this disease have been reported—eleven of them within the last three years. The first case was detailed by Niemann.[3] Knox, Wahl and Schmeisser [4] reported two more cases and Siegmund [5] still another. These writers believed that they were dealing with either Gaucher's disease or a condition closely allied to it in infants; Niemann erred in believing that his case satisfied the anatomic criteria necessary for a diagnosis of Gaucher's disease, but he saw clearly that the two conditions were quite different clinically. Mandlebaum and Downey [6] and Pick [7] pointed out the distinct anatomic and microchemical-

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*This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

1. Abt, A. F., and Bloom, W.: Essential Lipoid Histiocytosis (Type Niemann-Pick), J. A. M. A. **90**:2076 (June 30) 1928.

2. Bloom, W.: Splenomegaly (Type Gaucher) and Lipoid-Histiocytosis, Am. J. Path. **1**:595, 1925.

3. Niemann, A.: Ein unbekanntes Krankheitsbild, Jahrb. f. Kinderh. **79**:1, 1914.

4. Knox, J. H. M.; Wahl, W. H., and Schmeisser, H. C.: Gaucher's Disease: A Report of Two Cases in Infants, Bull. Johns Hopkins Hosp. **27**: 1, 1916.

5. Siegmund, H.: Lipoidzellenhyperplasie der Milz und Splenomegalie Gaucher, Verhandl. d. deutsch. path. Gesellsch. **18**: 58, 1921.

6. Mandlebaum, F. S., and Downey, H.: The Cases of Gaucher's Disease Reported by Drs. Knox, Wahl and Schmeisser, Bull. Johns Hopkins Hosp. **27**: 109, 1916.

7. Pick, L.: Der Morbus Gaucher und die ihm ähnlichen Erkrankungen, Ergebn. d. inn. Med. u. Kinderh. **29**:519, 1926; Ueber die lipoidzellige Splenohepatomegalie Typus Niemann-Pick als Stoffwechselerkrankung, Med. Klin. **39**:1, 1927.

histologic differences between Gaucher's disease and Niemann-Pick's disease. Pick suggested the name of lipoid cell splenohepatomegaly for Niemann's disease, and showed that it constituted a clinical and pathologic entity. In 1925, I reported three cases of this disease; one of these was diagnosed clinically by means of a splenic puncture and was verified by splenectomy. Since then, additional cases have been reported by Henschen,[8] Schiff,[9] Hamburger,[10] Corcan, Oberling, and Dienst,[11] Kramer,[12] Dienst and Hamperl,[13] Berman [14] and Abt and Bloom.[1] The latter case was diagnosed on purely clinical grounds and was verified by splenectomy.

THE CLINICAL PICTURE OF ESSENTIAL LIPOID HISTIOCYTOSIS

The reports of the fifteen cases on record show that this disease presents a striking clinical picture. The symptoms and the clinical courses in these cases were so similar and so distinctive that any one who is made familiar with them should recognize the condition readily. The outstanding clinical features of the condition have been repeatedly described during the past three years; hence I shall comment on them but briefly.

The condition in all of the cases started very early in life. In most of the cases, when the child affected had arrived at the age of 5 or 6 months, the parents noticed that its abdomen was unusually prominent and that the child was not progressing either mentally or physically. When brought to the attention of a physician, the child was found by him to have a great enlargement of the spleen and the liver and a peculiar, light yellow-brown coloration of the skin. As the patient grew older, the liver and the spleen became progressively larger, and the subcutaneous fat disappeared.

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8. Henschen, F.: Contribution a l'étude de la maladie de Gaucher, Comptes rendus du XII Congrès de médecine des Pays du Nord, Acta med. Scandinav., suppl. **16**:593, 1925.

9. Schiff, E.: Im Leben diagnostizierte lipoidzellige Splenohepatomegalie (Typus Niemann-Pick) bei einem 17 Monate alten Knaben, Jahrb. f. Kinderh. **112**:1, 1926.

10. Hamburger, R.: Lipoidzellige Splenohepatomegalie (Typus Niemann-Pick) in Verbindung mit amaurotischer Idiotie bei einem 14 Monate alten Mädchen, Jahrb. f. Kinderh. **116**:41, 1927.

11. Corcan, P.; Oberling, C., and Dienst, G.: La maladie de Niemann-Pick, Rev. franç. de pédiat. **3**:789, 1927.

12. Kramer, B.: Lipoid-Cell Splenohepatomegaly, Niemann-Pick Type: A Clinical Analysis of 9 Cases with a Report of One Additional Case, M. Clin. N. Amer. **11**:905, 1928.

13. Dienst, G., and Hamperl, H.: Lipoidzellige Splenohepatomegalie (Typus Niemann-Pick), Wien. klin. Wchnschr. **77**:1432, 1927.

14. Berman, S.: A Case of Lipoid-Histiocytosis, Am. J. Dis. Child. **36**:102 (July) 1928.

Even at the age of eighteen months these children could not recognize their parents nor could they sit up. Five of the fifteen children showed an amaurotic, hereditary idiocy. Several others were decidedly mongolian in appearance.

The children might gain somewhat in weight, but this was due, for the most part, to the enlargement of the liver and the spleen. These patients ran an interrupted low fever and usually harbored mild infections of the upper part of the respiratory tract. They became progressively more cachectic and died before their second birthdays.

In most of the cases, there was a mild anemia and a slight although definite leukocytosis. The blood showed a content of cholesterol, fatty acid and lecithin above the limits of the normal. The stools did not show anything abnormal. The urine was normal; I could not find any record of its being examined for its lipoid content. Hamburger found the functioning of the liver to be within normal limits, by means of tests with dextrose, levulose and indigo-carmine. He also found the blood sugar to be of normal amount.

The majority of the children with this disease were Jews.

MATERIAL

The present study of the disease is based on an examination of the organs from my series of four cases. Through the kindness of Dr. R. Jaffé, of the University of Illinois, I have studied, in addition, the tissues from the case reported by Berman. The material was obtained at autopsy, shortly after death, in three instances, and on surgical removal of the spleen and portions of a lymph node and of the liver in the other two. The tissues were fixed in a solution of formaldehyde and in the Zenker-formaldehyde solution. They were embedded in either paraffin or celloidin, serially sectioned and stained with hematoxylin and eosin, Mallory's anilin blue, iron hematoxylin and hematoxylin-eosin-azure II. Many of the latter preparations, which were first stained with Foot's silver impregnation method, gave particularly instructive pictures. Some small portions of the tissues were fixed by Champy's method.

Other portions of the various organs were stained also for the fats and the more complex lipoids with Weigert's iron hematoxylin, sudan III, nile blue sulphate and by the methods of Ciaccio and Lorraine-Smith-Dietrich; also by Fischler's technic for soaps. They were studied further for their iron content.

Scrapings of the spleen from our last case were studied with the supravital neutral red and janus green method.

GROSS EXAMINATION

The spleens in all the recorded cases of this disease were greatly enlarged. The smallest spleen (in case C of Bloom) weighed 62 Gm. and the largest (in Siegmund's case) weighed 310 Gm. The average of eight spleens was 168 Gm. The organ was yellowish pink, soft in consistency and slightly more yellow on section than externally. The malpighian bodies could be recognized as small, gray points, but, exclu-

sive of these, there was not a suggestion of the normal structure of the organ. Practically all of its substance had been replaced by a yellowish fatty material.

The liver was yellow and enlarged (in my case C it was not enlarged and was a light brown). It resembled in color the liver seen in cases of phosphorus poisoning and in cases of acute alimentary intoxication in children. The lymph nodes all over the body, might or might not be slightly enlarged; those of the mesentery might be bright yellow. There was usually a pale gray streaking of the pulmonary tissue, which was most marked under the pleura and along the broader septums; there was not, however, any definite consolidation. The other organs were without gross changes, except that the subcutaneous fat in late stages of the disease was greatly reduced in amount.

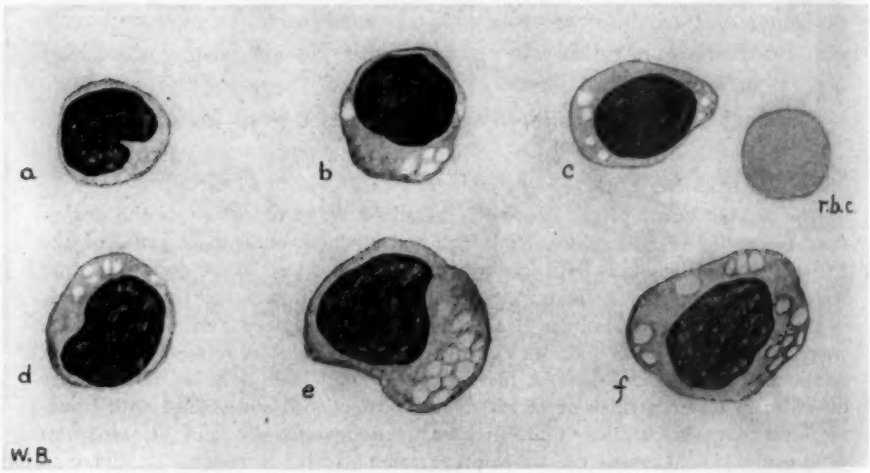

Fig. 1.—The letters *b*, *c* and *d* represent vacuolated lymphocytes in lipoid hystiocytosis; *e*, *f*, vacuolated monocytes; *a*, a lymphocyte as found in a dry smear of blood. The smear was prepared with the May-Grünwald-Giemsa stain. Leitz 2 mm. apochromatic objective and 15 × periplan ocular; camera lucida.

MICROSCOPIC EXAMINATION

Blood.—The count of the erythrocytes varied from 2,810,000 to 5,500,000 per cubic millimeter, averaging 4,300,000. The count of white blood cells varied from 10,400 to 29,200 per cubic millimeter, averaging 15,000. The proportions of the various leukocytes showed extreme variations in the different children and even in the same patient, from time to time. In the latest case, I paid particular attention to the appearance of the nongranular leukocytes as they were seen in dry May-Grünwald-Giemsa stained blood smears. On close examination,

I found that about 20 per cent of the agranulocytes contained peculiar large vacuoles. These occurred in both lymphocytes (fig. 1, *b, c, d*) and monocytes (fig. 1, *e, f*). The vacuoles varied somewhat in size; they were usually rounded, although some were oval or rod-shaped. They had sharp contours and seemed punched out of the cytoplasm. They were much larger and more conspicuous than the normal fine vacuolization of many agranulocytes (fig. 1, *a*). The vacuoles also occurred in cells which were difficult to classify as either lymphocytes or monocytes and which I consider to have been monocytoid lymphocytes. Some of the cells contained as many as twenty vacuoles. This vacuolization of the agranulocytes will be referred to again in the description of the sections of the lung. These vacuoles were usually more numerous and smaller than the so-called toxic vacuoles of the agranulocytes pictured by Nägeli and others. If this vacuolization is found to be a constant feature of future cases, it will certainly be a distinct aid in the diagnosis of this condition. I also saw an occasional leukocyte with one or two clear vacuoles.

Spleen.—In none of the spleens from the five cases that I examined, and in none of the remaining ten cases in the literature were there early stages of the involvement of this organ. In the cases thus far studied, the spleen showed only the well advanced changes, and in this respect differed from many of the other organs. With low power magnification, the organ was seen to be transformed into a huge mass of lightly staining cells. These were hard to delineate with hematoxylin and eosin. With hematoxylin-eosin-azure II (fig. 2) or with Mallory's aniline blue, the pale cells stood out as irregular cords between which were interspersed thin strands of darker staining cells. With higher power magnification, the pale cells were seen to be large and thoroughly vacuolated (fig. 5). The foam cells filled the pulp of the organ between the sinuses. The cells lining the sinuses (littoral cells) were rather low and were not vacuolated. The foam cells of the pulp had relatively large, irregular nuclei with some few heavy chromatin particles and one or more rather small nucleoli—they were the characteristic histiocyte nuclei (fig. 5). The fibers of the reticulum ran between the cells; in places they seemed to pass through the cell bodies, but this could not be determined exactly. Scattered through the pulp between the foam cells were a few large and small lymphocytes. With careful study, one could also find long, outstretched cells with dark nuclei in the pulp cords between the foam cells and close to the littoral cells of the sinuses. These were the resting mesenchymal cells from which some of the foam cells developed (fig. 5, *Emb*).

The malpighian bodies were usually widely separated from each other by the great masses of foam cells in the pulp cords. In sections stained with eosin-azure, under low power magnification, a varying number of

small clear areas were seen in the germinal centers (fig. 2). With higher magnification, these clear areas were seen to be large foam cells. They differed somewhat from the foam cells of the pulp cords. In the latter the vacuoles were rather small and fairly uniform in size; in the germinal centers, the vacuoles were few and showed great variations in size in a particular cell. In this location, the development of the foam cells from the embryonic reticular syncytium was readily demonstrable. The cells of the latter drew in their processes, their nuclei became larger and developed definite nucleoli, and at the same time vacuoles appeared in

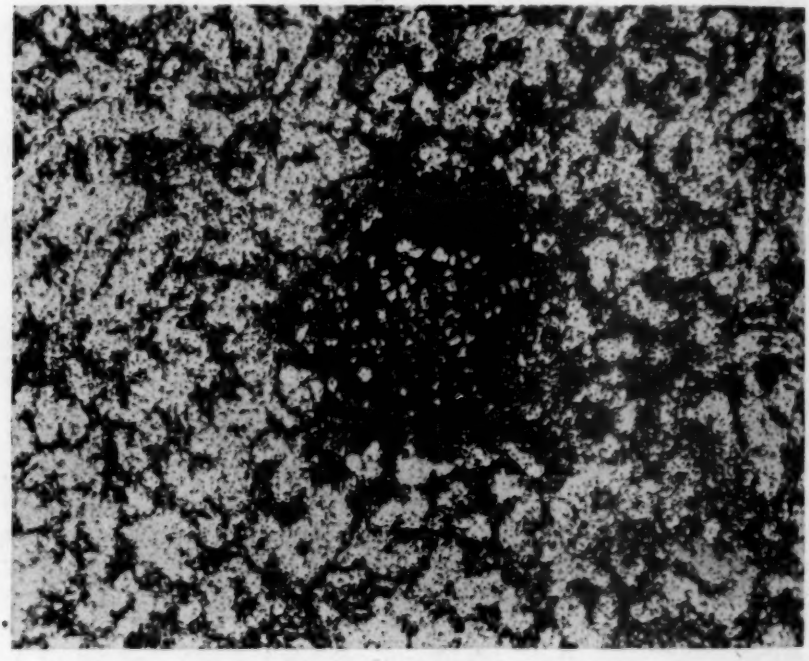

Fig. 2.—A section of a spleen as found in Niemann-Pick's disease. Foam cells may be seen in the pulp cords and in the malpighian body. The section was fixed in a formaldehyde-Zenker solution, and stained with hematoxylin-eosin-azure II; × 65.

the cytoplasm. These enlarged and many of them coalesced. The development of the foam cells from the embryonic reticular syncytium was hardly to be made out in the pulp cords; it was probable that early stages of this process in the spleen would have enabled one to follow this transformation. In neither the pulp nor the malpighian bodies was there any indication of the foam cells developing from lymphocytes; they arose exclusively from the mature histiocytes and from the embryonic reticular syncytium.

Interesting pictures were seen when the various stains for lipoids were applied to frozen sections of portions of the spleen fixed in formaldehyde solution. The foam cells did not stain positively with sudan III or nile blue sulphate. After mordanting of frozen sections in potassium bichromate, the cytoplasm of the foam cells was seen filled with granules, which stained bright yellow with sudan III and deep lavender with nile blue. In frozen sections, the foam cells stained deep blue black with Weigert's iron hematoxylin and by the method of Lorrain-Smith-Dietrich. Stained by these two meth-

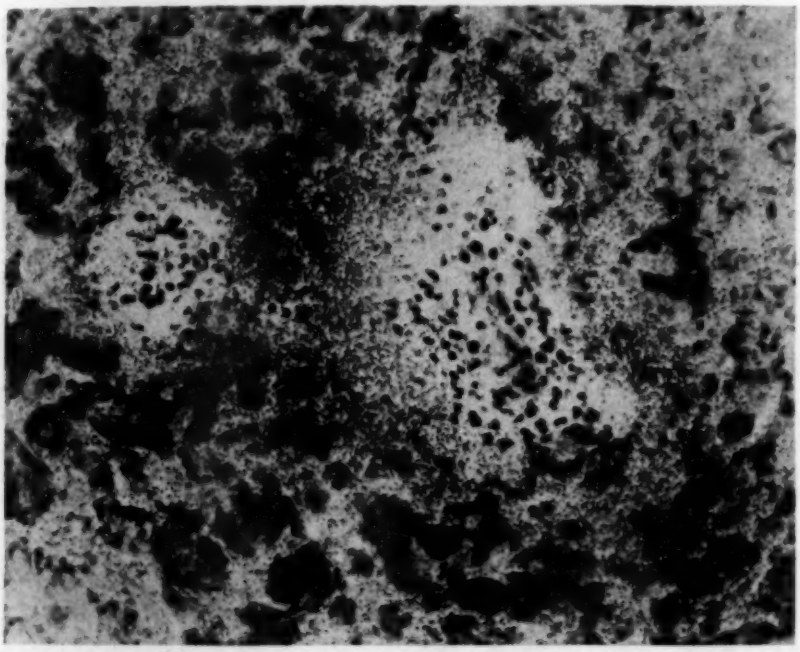

Fig. 3.—Cells containing lipoids, in the pulp and the malpighian body of a spleen as found in Niemann-Pick's disease. The lipoid is stained black by the Lorrain-Smith-Dietrich method; formaldehyde-fixed; × 65.

ods, the sections appeared like negatives when compared with the sections obtained by passing the tissues through alcohol, ether and xylol; for these solvents removed the lipoids and left the histiocytes as vacuolated shells. With these two methods of staining, the lipoid-laden cells in the germinal center stained much more quickly and darker than those of the pulp cords. The extreme differences in visible details between the sections of the spleen prepared by the usual histologic methods (fig. 2) and those prepared by the use of the Lorrain-Smith-Dietrich method (figs. 3 and 4) are apparent. The large cells in the spleen reacted negatively for iron.

In supravital smears of the splenic pulp stained with neutral red, the large cells were filled with small and large granules. These were of approximately the same size in a particular cell. They were dull, yellow gray, unstained, and varied from pink to light yellow when stained. As a rule, all of the granules or droplets in a given cell were of the same color. In these lipoid-laden macrophages, there were none of the bright yellow, orange and red vacuoles of various sizes which were so prominent in the histiocytes seen in the supravital films of the hematopoietic organs. A few of the peculiar pink or yellow vacuoles were

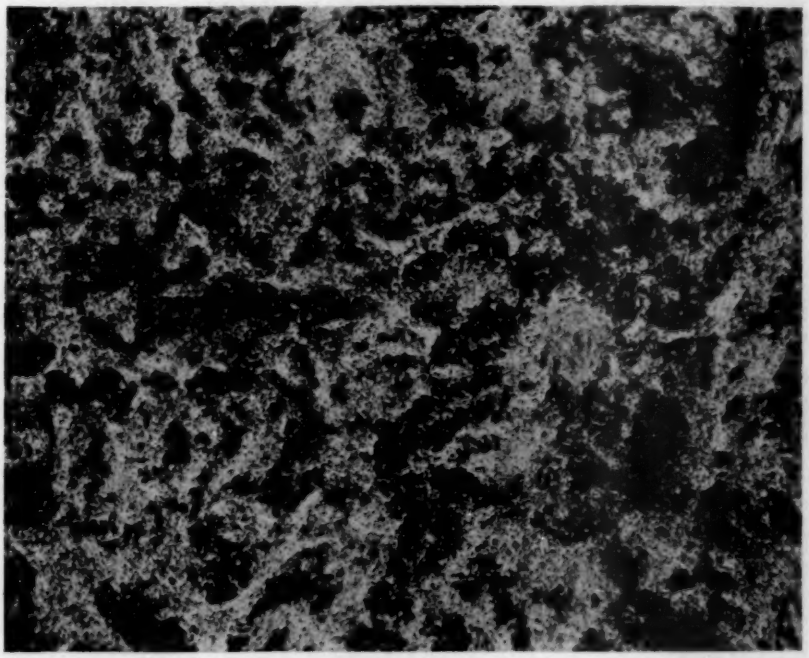

Fig. 4.—Another section of the same spleen, and prepared in the same way as in the section shown in figure 3.

contained, also, in some of the lymphocytes in these smears of the spleen. They were quite different from the orange, small, neutral red vacuoles of the agranulocytes and from the highly refractile, nonstaining lipoid droplets of the lymphocytes. These unusual droplets, which stained faintly with neutral red, corresponded with the vacuoles in the agranulocytes as seen in dry smears of the peripheral blood. Mitochondria stained green in the lymphocytes and the monocytes of the smears of the spleen, but were not seen in the large histiocytes because of the large neutral red granules in these cells.

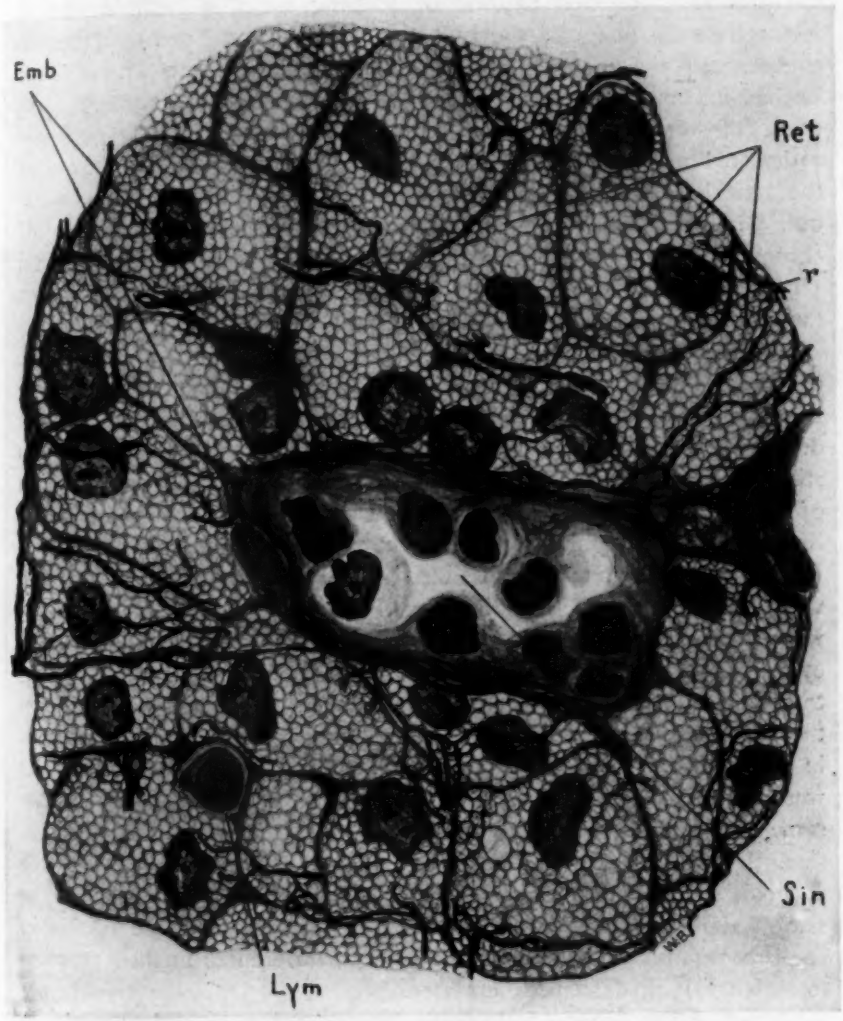

Fig. 5.—A drawing of a section of the spleen as it appeared in Niemann-Pick's disease. *Sin* represents the lumen of a sinus lined by nonvacuolated endothelial cells; *Emb*, the cells of the embryonic reticular syncytium; *Lym*, a lymphocyte; *Ret*, histiocytes of the pulp cords filled with vacuoles; *r*, a fiber of the reticulum. The section was fixed in formaldehyde-Zenker solution, silvered by Foot's method and stained with hematoxylin-eosin-azure II. Leitz 2 mm. apochromatic objective and 12 × periplan ocular; camera lucida.

Lymph Nodes.—The foam cells developed in the lymph nodes at a much later time than in the spleen. In every case examined, it was possible to find some lymph nodes which showed the early stages of the process. The best method of staining for this purpose was to impregnate the fibers of the reticulum by Foot's method and then to counterstain the material with hematoxylin-eosin-azure II. In the unchanged areas, the syncytial nature of the reticulum cells was beautifully demonstrated. The protoplasm was seen as a blue, finely reticulated mass about and occasionally through which the fibers of the reticulum passed. Intercellular membranes were not to be made out. The nuclei of the syncytium were dark and the particles of chromatin and the nucleoli were inconspicuous, especially with this stain (fig. 6, *a*). There were great numbers of free lymphocytes between the meshes of the cells of the reticulum.

As the syncytium was followed, one came to areas in which the cytoplasm of these cells increased in amount and became, at the same time, vacuolated. In relatively early stages, even when there was a high degree of vacuolization, the syncytial arrangement of these cells was still prominent (fig. 6, *b*). In other areas, in which the accumulation of lipoid droplets was still further advanced, the separation of individual lipoid filled histiocytes from the syncytium could be easily followed (fig. 7). Here completely isolated histiocytes (macrophages), filled with large vacuoles, with a distinct cell membrane and a single nucleus were prominent (fig. 7, *e*). There were also highly vacuolated masses of cytoplasm with three or four nuclei (fig. 7, *d*), which were beginning to round off from the hypertrophying syncytium (fig. 7, *a*, *b*, *c*). In the late stage of the transformation in the lymph nodes, the vacuoles in the histiocytes differed from those in the spleen. In the latter, as was pointed out, the vacuoles in a given cell were practically of the same size, and those of different cells showed but small variations in size. In the large foamy histiocytes of the lymph nodes, however, the vacuoles showed marked variations in size. These differences in the size of the vacuoles were possibly due to differences in the lipoids in the two organs, for there were some slight differences in their reaction to sudan III, as described hereafter.

Most of the fibers of the reticulum passed over and around the large foam cells. Occasionally, they passed directly through the body of a cell. This was unmistakable when the fiber passed through the entire depth of the cytoplasm close to the nucleus, as in the free macrophage *e* in the center of figure 7.

In none of the lymph node sections was there any indication of the transformation of lymphocytes into foam cells. Their origin from the resting or embryonic reticular cells was clear. Even in those areas which contained many large foam cells, one still found small, dark,

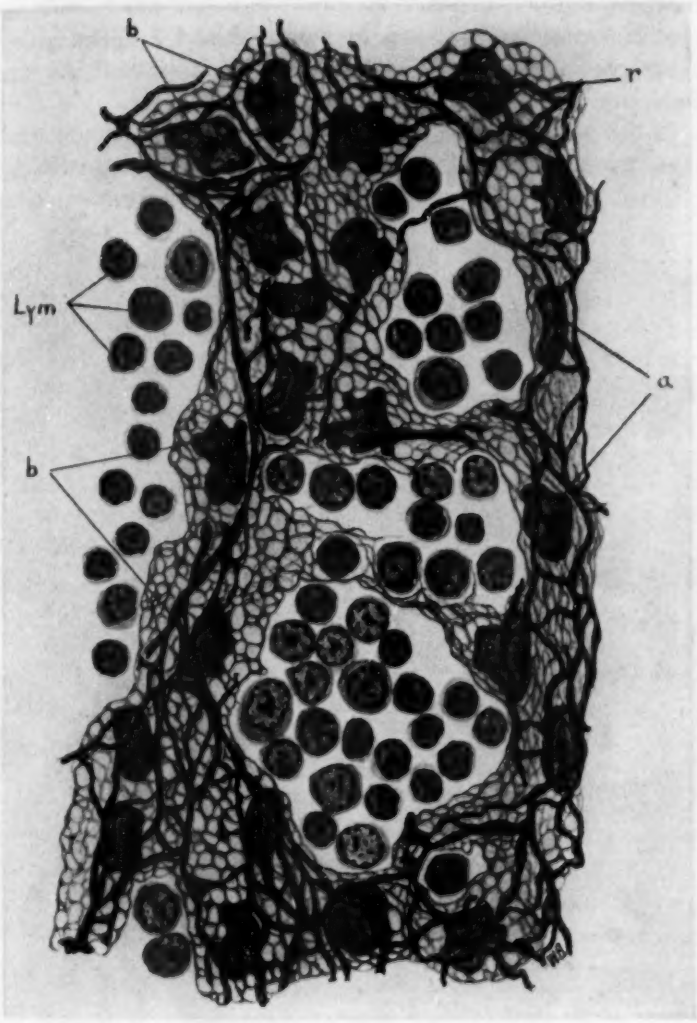

Fig. 6.—A drawing of a section of a lymph node as found in Niemann-Pick's disease; *a* represents cells of the reticular syncytium; *b*, similar cells which are becoming vacuolated; *Lym*, lymphocytes; *r*, a fiber of the reticulum. The section was fixed in formaldehyde-Zenker solution, silvered by Foot's method and stained with hematoxylin-eosin-azure II. Camera lucida and same optical system detailed for figure 5.

resting reticular cells—usually close to the fibers of the reticulum. From these, there was a complete series of cells showing progressive vacuolization and mobilization to the free lipoid filled macrophages. This process was clearly shown in figures 6 and 7. The histiocytes lining the medullary sinuses also became filled with lipoid droplets but they rarely broke free as vacuolated macrophages. In the later stages of the process in the lymph nodes, the embryonic reticular cells of the germinal centers also turned into the characteristic foam cells

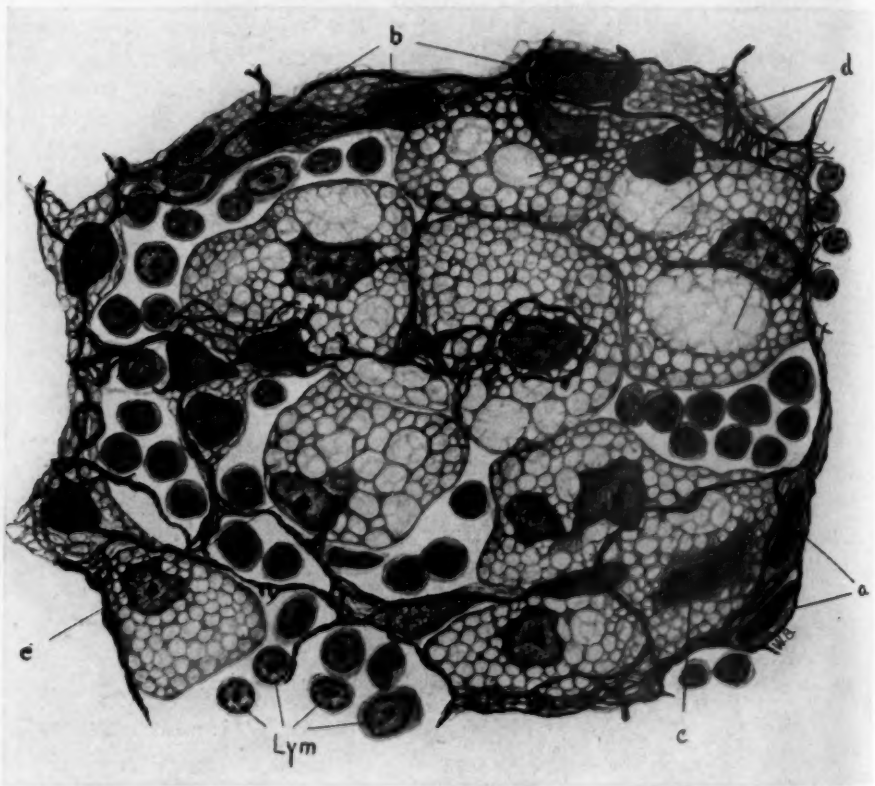

Fig. 7.—A drawing of a section of lymph node as found in Niemann-Pick's disease. *a* represents resting cells of the reticular syncytium; *b*, a first stage in the vacuolization of these cells; *c* and *d*, further stages in the vacuolization and rounding off of the reticular cells; *e*, highly vacuolated histiocytes, which are completely rounded; *Lym*, lymphocytes. The section was given the same preparation and the same optical system as have been detailed for figure 5.

in just the same way as they did in the germinal centers of the malphigian bodies of the spleen.

I did not see mitoses in the histiocytes containing the lipoids nor in any of the resting cells from which they developed. This could probably

be explained by the fact that the accumulation of the lipoids in the histiocytes extended over a long period of time, and consequently the mobilization of the histiocytes proceeded slowly.

The lymph nodes gave much the same reaction with the stains for the complex lipoids as the other organs did, except that there were a few cells which stained with sudan III without preliminary mordanting.

The lymphoid tissue of the gastro-intestinal tract underwent the same changes as that of the lymph nodes and the spleen. The histiocytes

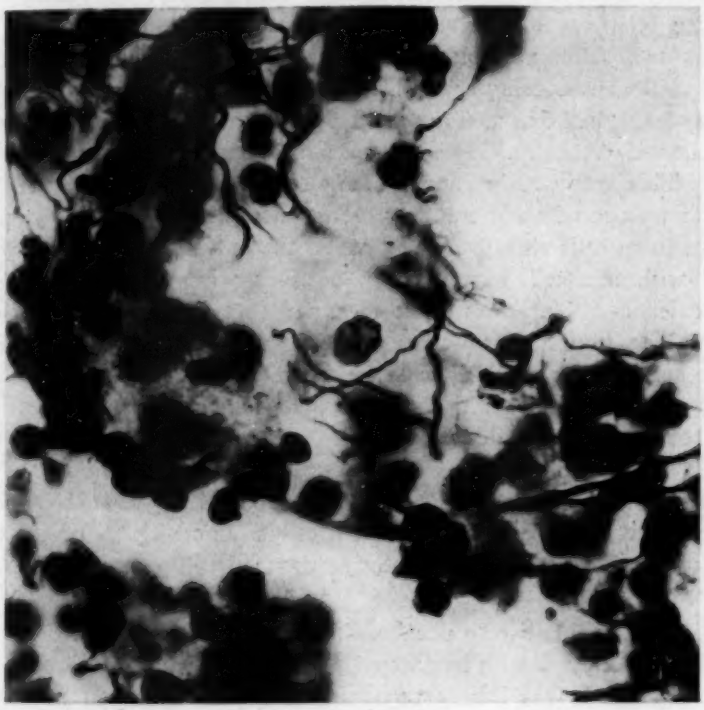

Fig. 8.—The bone marrow as seen under high power magnification in Niemann-Pick's disease. Several large foam cells may be found in the center of the field. Formaldehyde-Zenker solution was used for fixing the material; it was silvered by Foot's method, and the stain used was hematoxylin-eosin-azure II; × 1080.

became large and filled with lipoid droplets. The embryonic cells of the germinal centers of the follicles also became transformed into lipoid-containing macrophages.

Bone Marrow.—The bone marrow contained many foam cells (fig. 8). These were in the pulp of the tissue. Some of them were large, rounded, free cells; others were still in the reticular syncytium. The

origin of the foam cells from the latter was apparent and proceeded in much the same manner as was described in the case of the lymph node. In none of the marrows that I have examined were the foam cells as numerous and prominent as they were in the pulp cords of the spleen. The littoral cells of the sinuses of the bone marrow did not become foam cells.

Connective Tissue.—Sections of the connective tissue from beneath the skin as well as from that about and running through the various organs showed practically the same picture all over the body. The connective tissue always contained large numbers of foam cells. These had the relatively dark nuclei with coarse particles of chromatin and one or two small nucleoli, which were typical of the nuclei of the usual resting wandering cells (histiocytes). The foam cells here were usually somewhat flattened and their vacuolated cytoplasm might extend for unusual distances. The fibroblasts did not give rise to the foam cells. This was clearly shown in figure 9. The nuclei of the foam cells and those of the fibroblasts were quite different. The cytoplasm of the foam cells was prominent while that of the fibroblasts, as was usual with this cell, especially in locations with much collagen, was inconspicuous. Only by the most careful focusing could it be seen as a thin blue film in eosin-azure preparations. Moreover, transitional forms between the fibroblasts and the foam cells were absent. The latter developed from the local histiocytes. This sharp difference in participation between the fibroblasts and the histiocytes in the formation of the foam cells in the connective tissue was of some interest, for it afforded added evidence against the correctness of von Möllendorf's theory, that the fibroblast is the stem cell from which all of the other cells of the blood and the connective tissue develop (this is discussed later).

Liver.—As the disease developed, the liver became large. A section of the liver stained with hematoxylin and eosin, when the process was fully developed, was hardly recognizable as coming from this organ. All of the cells were highly vacuolated and pale. The characteristic arrangement of the liver cords and the sinusoids seemed at first glance, to be completely disrupted. There was, in late stages, an increase in the amount of periportal connective tissue. This was much less than was found in the marked cirrhosis of Gaucher's disease. With Mallory's aniline blue or with Foot's silver method combined with hematoxylin-eosin-azure II, the structure of the liver in this disease became apparent. With Mallory's stain, the liver cells were seen to be markedly vacuolated, with bits of dark red cytoplasm between the vacuoles. Between the cords of hepatic cells were great numbers of pale, blue gray foam cells. These were within, and frequently completely filled, the sinusoids. The

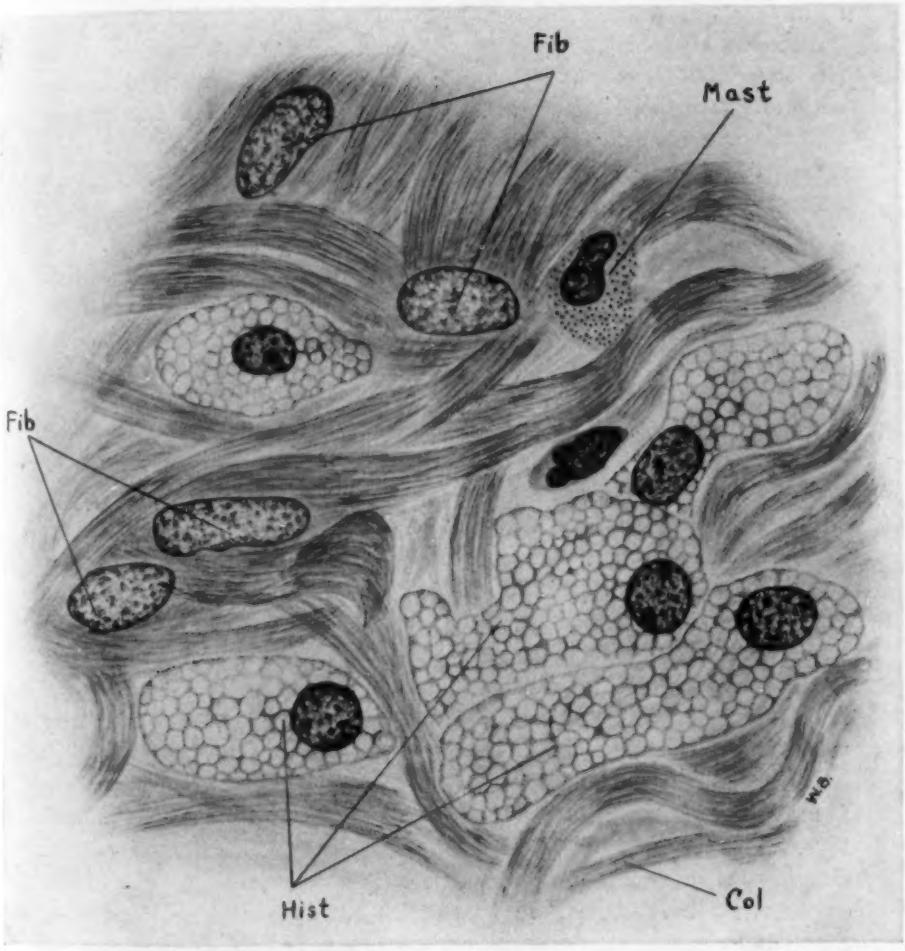

Fig. 9.—A drawing, highly magnified, of collagenous connective tissue from about the thymus. *Fib* represents fibroblasts; *Hist*, vacuolated histiocytes; *Mast*, a mast cell; *Col*, bundles of collagen. The material was fixed in formaldehyde-Zenker solution and stained with hematoxylin-eosin-azure II. Leitz 2 mm. apochromatic objective and 12× periplan ocular; camera lucida.

foam cells appeared much like those in the lymph nodes described. The vacuoles had sharp edges and varied considerably in size. Thus they differed from the vacuoles of the cells of the liver cord; these had hazier outlines and showed a greater tendency toward confluence. The foam cells were intravascular and all stages of their development from the resting endothelial cells through the Kupffer cells were plentiful. Even when all of the sinusoids in a given area were greatly distended with the vacuolated histiocytes, one could always find a few resting endothelial cells. These had thin streamers of cytoplasm and were rather inconspicuous because of the fibers of the reticulum which surrounded them (fig. 13).

But the clearest pictures were to be seen in the earlier stages. The liver of case *C*, reported in my first paper, showed the earliest stages in the development of the foam cells. Here it was easy to trace the progressive vacuolization of both the epithelial cells and the cells of the connective tissue in this organ. The Kupffer cells enlarged first and became greatly vacuolated, the vacuoles showing extreme variations in size (fig. 11, Kc). Then the endothelial cells began to enlarge and became vacuolated. These changed into foam cells just as the Kupffer cells did. The epithelial cells of the liver became vacuolated, the vacuoles first appearing in that portion of the hepatic cell closest to the biliary capillary. Later, the entire cytoplasm became vacuolated. These stages are shown in figure 11. The vacuoles of the hepatic cells were much more irregular than those of the Kupffer cells.

Most of the great increase in size of the liver was due to the accumulation of lipoids in the endothelial cells. Many of the hepatic cells became larger because of droplets of lipoids, but, as a rule, they were only slightly larger than the normal hepatic cells.

Corcan, Oberling and Dienst had some difficulty in separating the foam cells arising from the hepatic cells and those from the endothelial cells. There should have been no difficulty in this, as I have pointed out, because of the difference between the two types of cells in the character of the vacuoles. Moreover, with Mallory's aniline blue stain, the cytoplasm between the vacuoles was dark red in the hepatic cells and pale blue in the endothelial cells. When Eppinger's stain for bile capillaries was used, the grouping of the hepatic cells about the bile capillaries, was also helpful in discriminating between the two types of foam cells. In addition, the nuclei of the hepatic cells were practically always round, pale, and provided with one or two conspicuous nucleoli. The nuclei of the Kupffer cells were irregular in outline because they conformed to the lipoid vacuoles. They had heavier particles of chromatin and less prominent nucleoli. Pick, also, did not find any difficulty in separating the two types of cells in the liver. He, too,

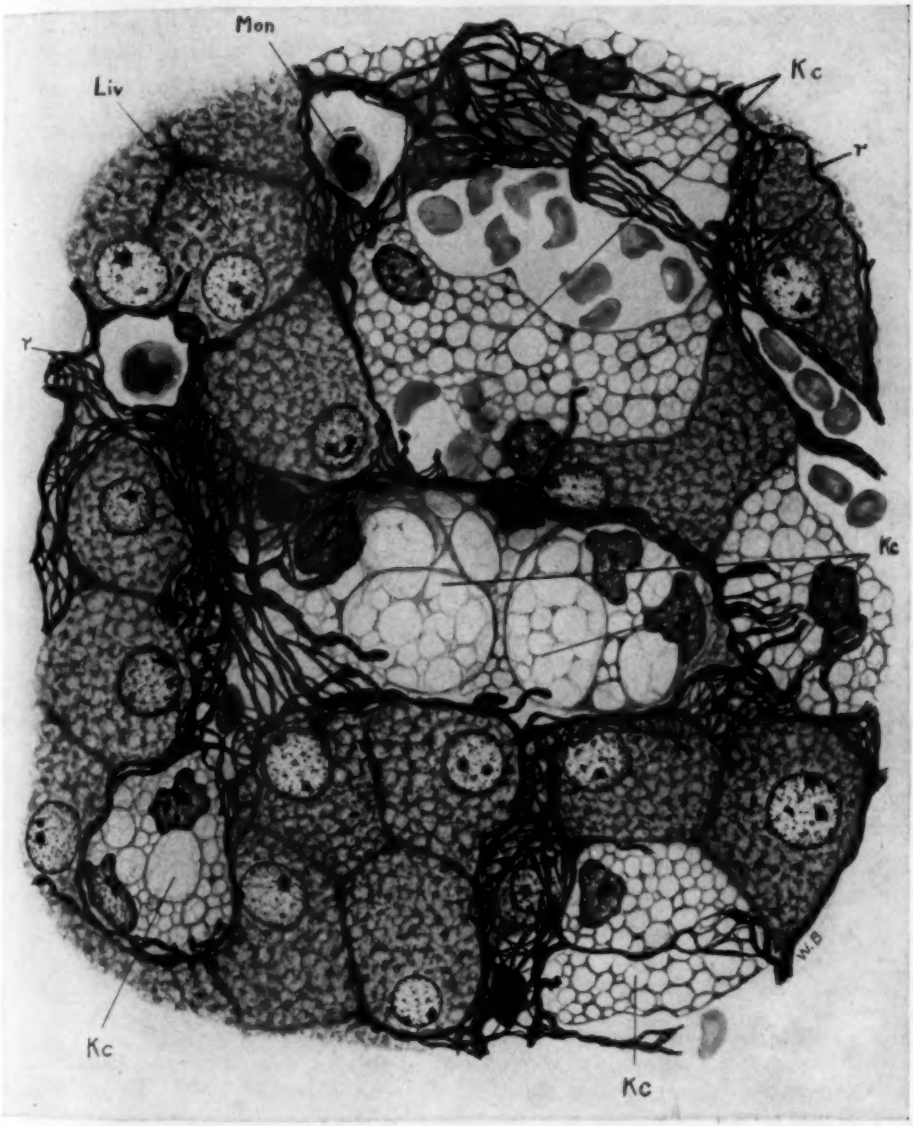

Fig. 10.—A drawing of a section of the liver as found in the late stages of Niemann-Pick's disease. *Liv* represents vacuolated hepatic cells; *Kc*, vacuolated Kupffer cells; *Mon*, a monocyte; *r*, a fiber of the reticulum. The material was fixed in formaldehyde-Zenker solution, silvered by Foot's method and stained with hematoxylin-eosin-azure II. Same optical system as described for figure 9; camera lucida.

derived the foam cells in the sinusoids from both endothelial and Kupffer cells.

With the use of the special stains for lipoids, the intravascular foam cells stood out prominently against the hepatic cells. The former were black, when stained by the Lorrain-Smith-Dietrich method, while the epithelial cells contained a varying number of round or rod-shaped black

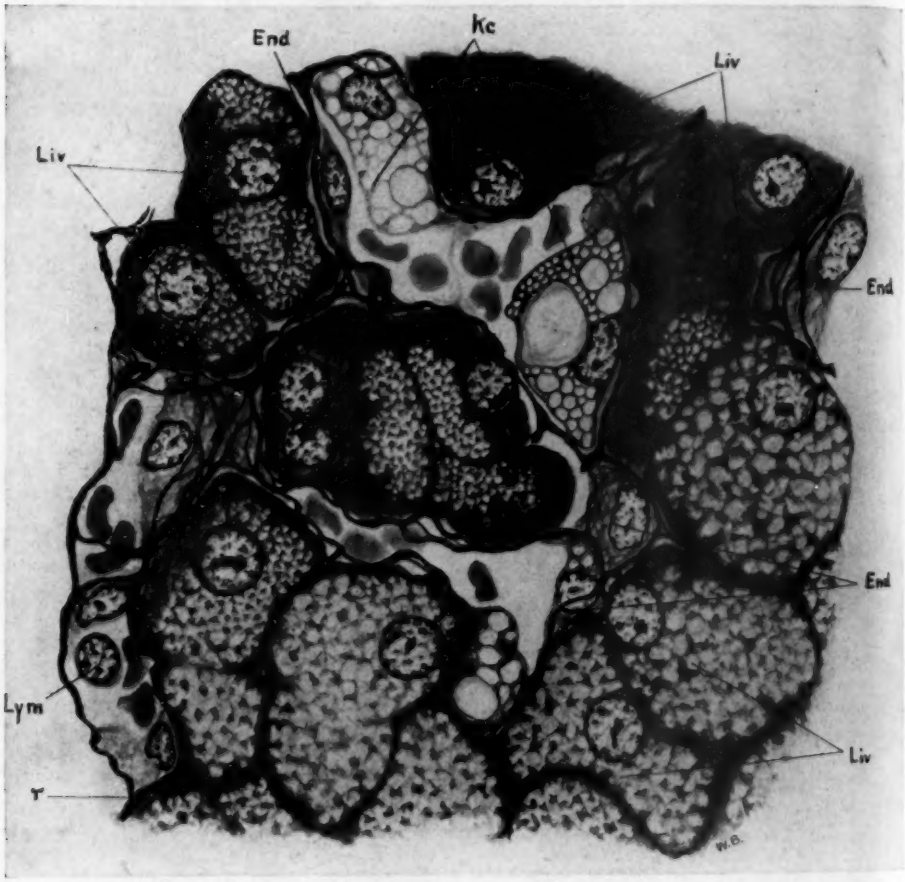

Fig. 11.—A drawing of a section of a liver as seen in an early stage of Niemann-Pick's disease. *End* represents endothelial cells in the early stages of vacuolization or without vacuoles; the other abbreviations and the optical system correspond with those in figure 10. Mallory's aniline blue stain was used. The early vacuolization of some of the hepatic cells may be seen, as well as of the endothelial and Kupffer cells.

dots. In such preparations, it was clear that the greater part of the vacuolization of the epithelial cells of the liver was not due to stored lipoid; it was probably due to accumulation of glycogen. This difference

between the hepatic and the intravascular foam cells in their reaction to the stains for lipoids was pointed out in my first paper and was pictured by Pick (fig. 3, 7b).

Suprarenal Gland.—The medulla was greatly thickened by a layer of foam cells. This, in places, was divided into two broad layers, which separated the medullary cells from those of the zona reticularis of the cortex (fig. 12). The littoral (reticulo-endothelial) cells of the cortex were occasionally changed into foam cells, but these were much less numerous than one would have expected. In material prepared by the

Fig. 12.—A section of a suprarenal gland as found in Niemann-Pick's disease. The medulla is greatly thickened with two layers of foam cells, which appear pale. This representation may be contrasted with that in fig. 13. The material was fixed in formaldehyde solution and stained with hematoxylin-eosin; × 65.

Lorrain-Smith-Dietrich method, with low power magnification, the medulla was seen to be a mass of closely packed, black granules and it stood out sharply against the cortex, which contained but a few scattered cells containing lipoids. Occasional small groups of cortical cells in the medulla did not react positively to the stain for lipoids and were accordingly prominent (fig. 13). In sections treated by silver impregnation, one saw clearly that the argentophile cells did not become vacuolated and did not turn into foam cells.

The foam cells of the medulla were similar to those described as occurring in the hematopoietic organs. They were definitely extravascular. In all of the suprarenal glands I examined and in those described in the literature dealing with this disease, the foam cells were so numerous that it was impossible to determine their exact origin. This point can only be settled by obtaining suprarenal glands at a much earlier period in the course of the disease.

Thymus.—The thymus was greatly changed, although not enlarged in Niemann-Pick's disease. In the thymus of my case *C*, from a boy

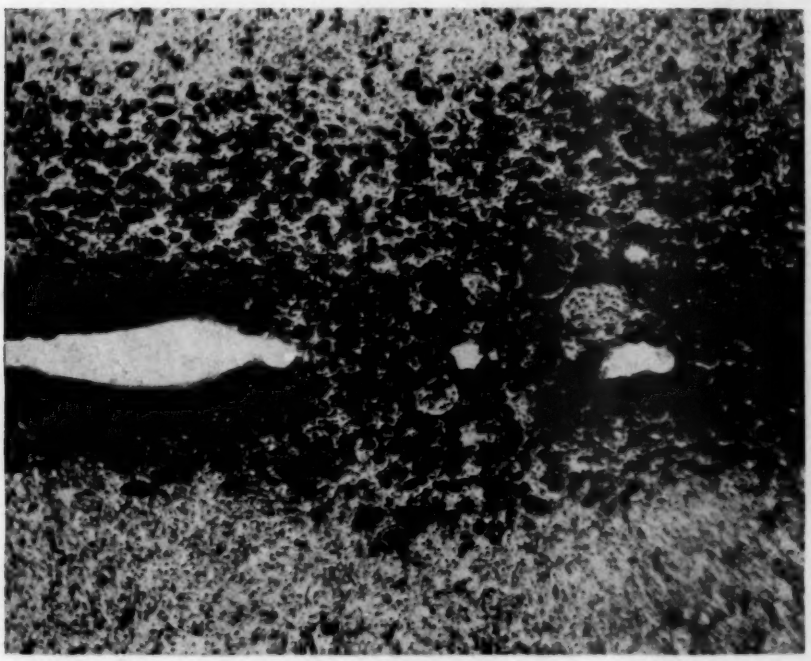

Fig. 13.—A section of the same suprarenal gland as shown in figure 12. The lipoid material is stained black. The absence of lipoid in the cortex should be noted. Formaldehyde was used for the fixation together with the Lorrain-Smith-Dietrich method of staining; × 65.

who died of meningitis at the age of 7 months, most of the normal structure was replaced by great masses of foam cells (fig. 14). At first sight, it was difficult to identify the organ; closer examination revealed Hassal's bodies. The collagen bundles of the interlobular connective tissue were heavily infiltrated with foam cells. These had the typical histiocyte nuclei standing out sharply against those of the nearby fibroblasts. The latter had typical large, oval, flat nuclei with a relatively thin nuclear membrane and dustlike particles of

chromatin. It was difficult to see the cytoplasm of the fibroblasts; with eosin-azure II, it was a coarsely reticulated, thin, blue film. Indications of the fibroblasts turning into foam cells were absent.

In slides silvered by Foot's method, the foam cells of the interlobular connective tissue were free of silver dust. The foam cells in the gland, however, were frequently heavily sprinkled with small black granules of silver. The foam cells were most frequent in the cortex and practically replace it in all my material. They occurred, in some places, as single cells between the fibers of the reticulum, while, in

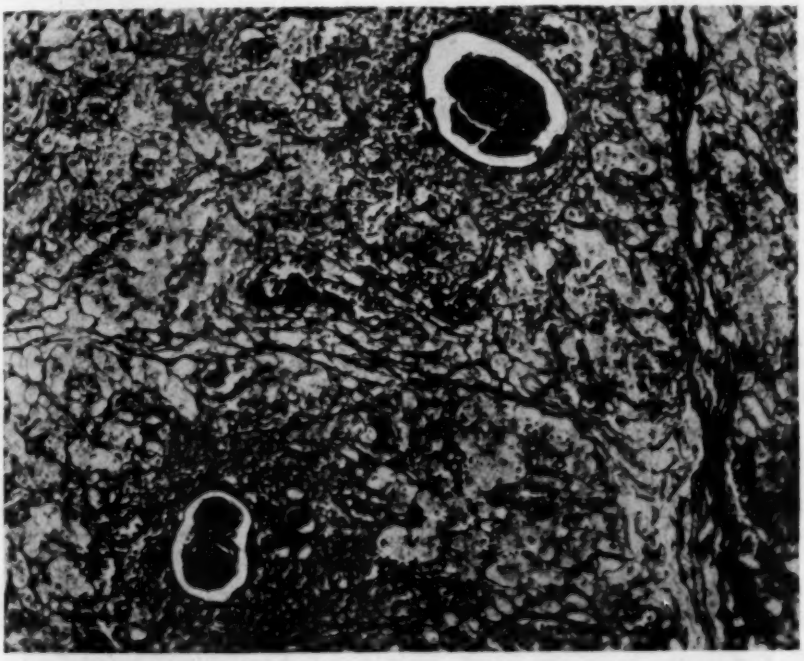

Fig. 14.—A section of the thymus as seen in Niemann-Pick's disease. Large masses of foam cells are encroaching on the medulla. Two badly distorted Hassal's bodies are surrounded by unaffected tissue. The material was fixed in formaldehyde-Zenker solution, and after treatment by Foot's method, stained with hematoxylin-eosin-azure II; × 150.

others, they formed small nests surrounded by the fibers. They were also numerous in the medulla and were closely packed in places, about Hassal's bodies.

The foam cells of the thymus had two kinds of nuclei. In one group, the nuclei were relatively small, dark and wrinkled; these were the characteristic histiocyte nuclei. In the other group, the nuclei were somewhat larger, rounded, rather vesicular, and contained little

chromatin and a conspicuous small nucleolus. They were highly suggestive of the nuclei of epithelial cells. Because of the extensiveness of the foam cell change in the thymus, it was difficult to trace them to the epithelial reticulum and almost as hard to connect them with the mesenchymal reticulum cells.

In material stained by Mallory's method or prepared with silver solutions, in which the smaller blood vessels were conspicuous, the foam cells were seen arranged in large groups about the vessels. This position spoke strongly in favor of their origin from the embryonic perivascular mesenchyme, which Tschassownikow [16] and Popoff [16] showed is plentiful

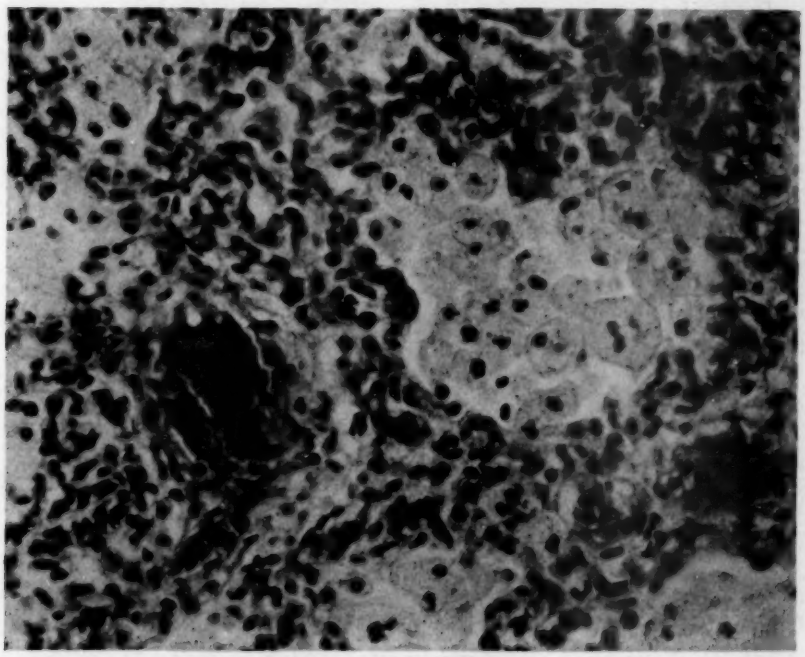

Fig. 15.—A section of a lung from a patient dying of Niemann-Pick's disease. Large masses of foam cells fill the alveolar spaces. The material was fixed in formaldehyde-Zenker solution and stained with hematoxylin-eosin-azure II; × 350.

in the pulp and the interlobular tissue of the thymus. However, the origin of some, at least, of the foam cells from the cells of the epithelial reticulum was highly probable. The final answer to this problem awaits a better elucidation of the structure of this organ as well as the opportunity of studying it in very early stages of this disease.

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15. Tschassownikow, N.: Ueber die in vitro-Kulturen des Thymus, Arch. f. exper. Zellforsch. **2**:250, 1926.

16. Popoff, N. W.: The Histogenesis of the Thymus as Shown by Tissue Cultures, Arch. f. exper. Zellforsch. **4**:395, 1927.

Lung.—The septums and the alveolar spaces of the lung contained great numbers of foam cells. In some alveoli, all of the air space was filled with these large cells. Some of them were multinucleated (fig. 15). They reacted positively to the stains for lipoids. In my first paper, I pointed out that there were many vacuolated free cells in the lumen of the pulmonary capillaries and larger vessels. I ascribed a histiocytic (Kupffer cell) origin to these cells, comparable with the pictures seen in heavily vitally-stained animals. After restudying my material, it was clear that many of the foam cells within the capillaries were small and had the typical nuclei of lymphocytes and monocytes. (In this connection, fig. 16, *Lym* and *Mon,* may be compared with fig. 1). There could not be any question that the circulating agranulocytes developed large vacuoles in their cytoplasm and turned into larger, lipoid laden, intravascular macrophages (fig. 16, *Mac*); for there was a complete series of transitional forms between the small and the large foam cells. The foam cells on the alveolar walls and in the air spaces could be traced to the septum cells of Lang[17] (fig. 16, *Sep.*). These were inconspicuous cells with little cytoplasm and rather small nuclei, which were tucked away within the septums. According to Lang, they are embryonic, mesenchymal cells, which under certain stimuli, such as explantation, can differentiate into phagocytes or histiocytes. His plates show developing vacuolated septum cells in rabbit lung cultures similar to those seen in the lungs in Niemann-Pick's disease. In my own work with cultures of the rabbit's lung,[18] I was convinced of the mesenchymal origin and nature of the septum cells. Gardner and Smith,[19] in their recent study of the origin of the alveolar phagocyte, agreed with Lang. The nonparticipation of the pulmonary capillary endothelium in the formation of the vacuolated macrophages is clearly shown at *End* in figure 16. The question as to the function and the fate of the alveolar epithelium is still unsettled.

Epithelium.—The epithelial cells of practically all of the organs—except those of the gastro-intestinal tract—showed marked changes when the disease was well advanced. The vacuolization of the hepatic cells has already been described.

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17. Lang, F. J.: The Reaction of Lung Tissue to Tuberculous Infection in Vitro, J. Infect. Dis. **37**:430 (Nov.) 1925; Ueber Gewebskulturen der Lunge, Arch. f. exper. Zellforsch. besonders Gewebezuchtung (Explantation) **2**:93, 1926.

18. Bloom, W.: Immune Reactions in Tissue Culture: I. Reaction of Lungs from Normal and Immunized Rabbits to Pigeon Erythrocytes, Arch. Path. **3**: 608 (April) 1927.

19. Gardner, L. U., and Smith, D. T.: The Origin of the Alveolar Phagocyte Studied in Paraffin Sections of Tissue Stained Supravitally with Neutral Red, Am. J. Path. **3**:445, 1927.

Thyroid.—The epithelium of this gland became highly vacuolated; when stained for the complex lipoids, it reacted very positively. The cytoplasm was not increased in amount; in most of the acini it was rather low. The nuclei of the epithelium were round or oval and somewhat vesicular, and were not indented. The colloid was of normal appearance. The gland had little stroma; this, in its thickest parts, contained a few vacuolated histiocytes. The fibroblasts remained

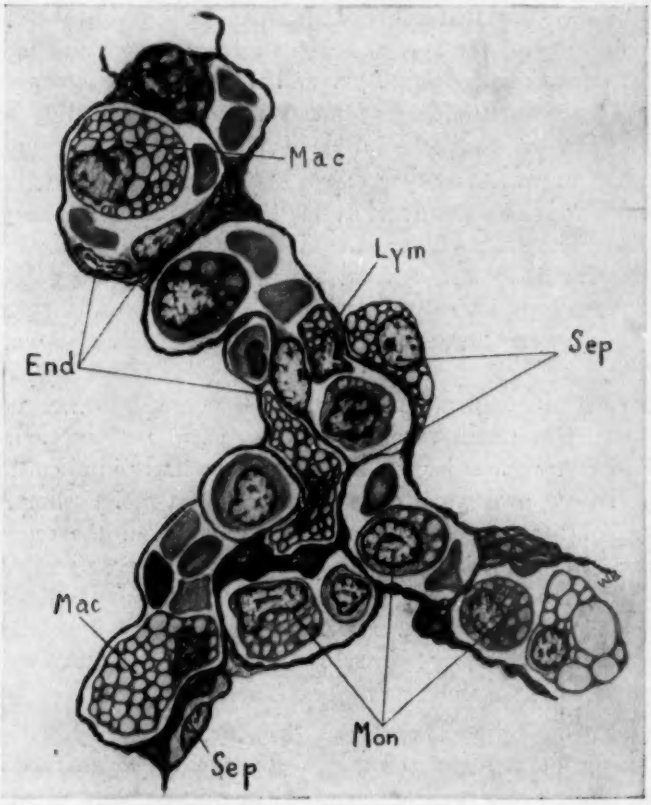

Fig. 16.—A drawing of a section of an alveolar septum of a lung as found in Niemann-Pick's disease. *End* represents endothelial cells; *Mac,* macrophages; *Mon,* vacuolated monocytes; *Lym,* a vacuolated lymphocyte; *Sep,* septum cells, resting and mobilizing. The material was fixed in formaldehyde-Zenker solution and stained with Mallory's aniline blue. Leitz 2 mm. apochromatic objective and 12 × periplan ocular; camera lucida.

unchanged; they did not turn into foam cells. The capillary endothelium might be slightly vacuolated, but the cells never became large and evidence of their turning into large, highly vacuolated foam cells was

lacking. The vacuolization of the thyroid epithelium and the endothelial cells of the capillaries in this gland (fig. 17) afforded a striking contrast with the histiocytic foam cells in the hematopoietic organs, described and pictured in the preceding pages.

One section through the thyroid passed through the parathyroid. Most of the cells in this gland contained one or two fairly large vacuoles. The cells were not increased in size above the normal. I did not test this gland with any of the stains for lipoids.

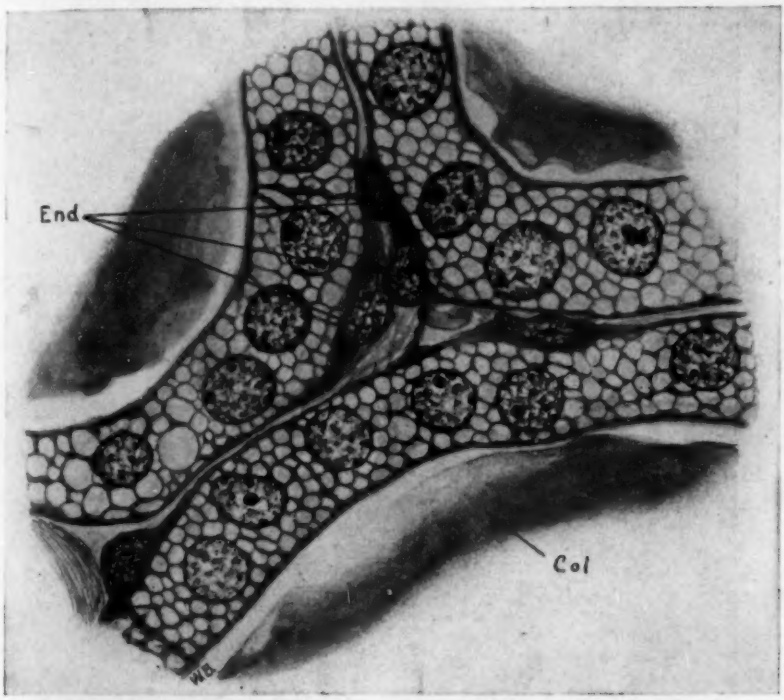

Fig. 17.—A drawing of a section of the thyroid as found in Niemann-Pick's disease. The epithelium is diffusely vacuolated. The endothelial cells (*End*) are practically unchanged. *Col* represents colloid. The material was fixed in formaldehyde-Zenker solution and stained with hematoxylin-eosin-azure II. Leitz 2 mm. apochromatic objective and 12 × periplan ocular; camera lucida.

Pancreas.—The exocrine cells of the pancreas become vacuolated during the course of this disease. In material stained with eosin-azure II, the secretion granules were bright red and were clustered at the proximal—that is, the lumen side of the cells, while the vacuoles appeared between the nucleus and the basement membrane. The vacuoles were rather small, rather regular and sharp in outline and

suggested those of the thyroid epithelium. The endocrine cells also developed numerous small vacuoles. In the interlobular connective tissue were a few vacuolated histiocytes.

Kidney.—With the routine hematoxylin and eosin stain, some vacuolization of the tubular epithelium could be seen and occasional scattered foam cells in some of the glomeruli (fig. 18). The Mallory stain accentuated the vacuolization. With the stains for lipoids, droplets of lipoid were seen in these locations as well as in the capillary endo-

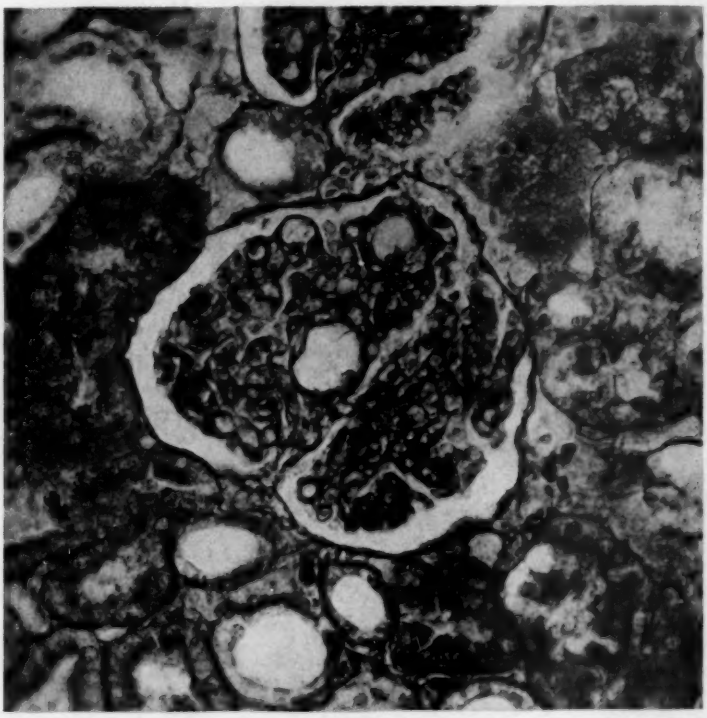

Fig. 18.—A section of the kidney as found in Niemann-Pick's disease. Two large and one small foam cell may be seen in the glomerulus. The section was fixed in formaldehyde-Zenker solution, and stained with Mallory's aniline blue; × 350.

thelium and diffusely as fine droplets throughout the glomeruli. It was difficult to determine the origin of the foam cells in the glomeruli (see Comment). Foam cells of relatively small size were found in the arteries of the kidney.

Lipoid droplets accumulated also in the cells of the anterior lobe of the hypophysis and in the glia cells of the posterior lobe. They were to be found in the tracheal and the bronchial epithelium as well

as in that of the mucosa of the fallopian tubes and the uterus. The droplets of lipoids in these various epithelial cells must be considered as being the result of a process different from the accumulation of lipoids in the histiocytes, for the epithelial cells did not increase progressively in size as they held more and more of the lipoids; the histiocytes, on the other hand, became large because of the lipoid inclusions.

The Lorrain-Smith-Dietrich method revealed that there were also droplets of lipoids in the cartilage cells and in the ganglion cells of the brain as well as in those of the retina (Pick). They occurred also in the glia cells of the central nervous system, in the sympathetic ganglion cells and in the sheaths of the medulated nerves. They might be numerous, also, in the smooth, cardiac and striated muscle cells. Foam cells of histiocytic origin were conspicuous between these various muscle cells.

Small accumulations of the characteristic foam cells were to be found likewise beneath the endothelium of the larger arteries and veins. In such locations, they were always covered by the flattened, resting endothelium, and there was no suggestion of the origin of the foam cells from the endothelium.

The Stored Lipoids in Niemann-Pick's Disease.—The reactions of the sections of the organs in this disease to the various methods of staining that were carried out indicated that the lipoids present were probably lecithin. This tallied well with the results of the chemical examinations of the spleen which had been made by Wahl and Richardson,[20] Siegmund,[5] Bloom and Kern,[21] Brahm and Pick,[22] Kramer [12] and Corcan, Oberling and Dienst.[11] All of these writers found a great increase in lecithin and, to a less extent, in cholesterol, in the spleen. Similar observations were also reported for the liver by Wahl and Richardson and Corcan, Oberling and Dienst.

Miss Kern and I were able also to corroborate the work of Epstein [23] and Lieb [24] to the effect that the stored material in Gaucher's disease is kerasin. This agrees with the results of the microchemical histologic

20. Wahl, H. R., and Richardson, M. L.: A Study of the Lipin Content of a Case of Gaucher's Disease in an Infant, Arch. Int. Med. **17**:238 (Feb.) 1916.

21. Bloom, W., and Kern, R.: Spleens from Gaucher's Disease and Lipoid-Histiocytosis: The Chemical Analysis, Arch. Int. Med. **39**:456 (March) 1927.

22. Brahm, B., and Pick, L.: Zur chemischen Organanalyse bei der lipoidzelligen Splenohepatomegalie Typus Niemann-Pick, Klin. Wchnschr. **6**:267, 1927.

23. Epstein, E.: Beitrag zur Chemie der Gaucherschen Krankheit, Biochem. Ztschr. **145**:398, 1924.

24. Lieb, H.: Cerebrosidspeicherung bei Morbus Gaucher, Ztschr. f. phys. Chem. **140**:305, 1924; **170**:60, 1927.

examination in Gaucher's disease; for the large cells in that disease did not react positively to any of the stains for lipoids.

COMMENT

In the preceding pages it has been shown that there is a great increase in phosphatids and, to a lesser extent, in cholesterol in practically all of the cells and tissues (including the blood) in essential lipoid histiocytosis. Morphologically, this is manifested by a great accumulation of droplets of lipoid in the phagocytes all over the body and to a much less degree in the epithelial cells of most of the glands, and in the different types of muscle, cartilage, ganglion and glia cells. Some of the vascular endothelial cells also contained droplets of lipoids.

The presence of the phagocytes containing lipoids all over the body is in many ways comparable to the pictures seen in tissues of animals when foreign particulate matter or vital dyes have been injected experimentally. In much the same way as these experiments threw light on the relationships between certain of the cells of the connective tissue in the animals, so the study of the organs in Niemann-Pick's disease has helped to elucidate similar cell relationships in man.

When a vital dye such as lithium carmine or trypan blue is injected into an animal, there ensues, during the first few hours, a diffuse staining of all of the tissues (Teploff [25]). By the end of the first day, the dye appears as granules in certain of the cells of the connective tissue. These are the cells of the pulp and of the littoral reticulum of the spleen, the lymph nodes and the bone marrow, the Kupffer cells of the liver and the resting wandering cells of the connective tissue. In heavily stained animals, the fibroblasts, also, all over the body, contain a few fine granules of the dye. The epithelial cells of the liver and of the renal tubules contain much of the dyestuff. The vascular endothelium does not take up either lithium carmine or trypan blue. After the intravenous injection of India ink, the vascular endothelium contains much of this material but it is soon transmitted to the perivascular phagocytes (Lang,[26] Stilwell [27]).

A somewhat similar process occurs in essential lipoid histiocytosis. From the few analyses which have been made of the blood of children with this affection, it is clear that the lipoid content of the blood

25. Teploff, J.: Ueber den Entwicklungsgang der vitalen Karminspeicherung im Organismus, Ztschr. f. d. ges. exper. Med. **45**:548, 1925.

26. Lang, F. J.: Rôle of Endothelium in the Production of Polyblasts (Mononuclear Wandering Cells) in Inflammation, Arch. Path. **1**:41 (Jan.) 1926.

27. Stilwell, F.: On the Phagocytic Capacity of the Blood Vessel Endothelium of the Frog's Tongue and Its Presumed Transformation into Wandering Cells, Folia haemat. **33**:81, 1926.

is increased above the normal. This lipoidemia probably starts at an extremely early age, for the disease is well advanced when the children are 5 or 6 months old. The connective tissue phagocytes have become large and are filled with lipoids. Most of the epithelial cells also contain the droplets of lipoids, but do not enlarge and do not turn into the free foam cells as the histiocytes do. That is, the epithelial, muscle and ganglion cells containing the droplets of lipoid may be considered as undergoing a lipoid degeneration. The reaction of the phagocytes is different from this, for, as was stated in the descriptions of the various organs, the mesenchymal reserves of the different tissues are mobilized to produce the large histiocytic cells, which are filled with lipoids.

In studying the tissues for the source of the lipoid-laden phagocytes, certain genetic relationships between some of the various connective tissue cells of the human organism become apparent. All of the stages in the mobilization of the embryonic mesenchymal cells (Maximow [28]) and the mature histiocytes, as well, into large, lipoid-containing histiocytes can be seen with great clarity. The foam cells develop from the resting endothelial and Kupffer cells in the liver, from the reticulum cells of the lymph nodes, the reticulum cells (exclusive of the littoral cells) in the spleen and the bone marrow, from the reticulum cells of the medulla of the suprarenal gland, from the septum cells of the lung, from the resting wandering cells of the connective tissue and the perivascular embryonic cells and phagocytes all over the body.

The intravascular agranulocytes also develop into lipoid filled macrophages in the lung and the kidney. The transformation of these cells into the macrophages agrees well with the observations of Maximow [29] in inflamed tissues and leukocytic cultures [30] from the blood and with the results of my experiments in which I used cultures of the lymphocytes of the thoracic duct of the rabbit.[31] In all this work, the ability of the lymphocytes to turn into macrophages and later into fibroblasts was clearly demonstrated.

The development of the foam cells from both histiocytes and blood lymphocytes is the same process that Maximow described for the origin

28. Maximow, A.: Ueber undifferenzierte Blutzellen und mesenchymale Keimlager im erwachsenen Organismus, Klin. Wchnschr. **5**:2193, 1926.

29. Maximow, A.: Experimentelle Untersuchungen über entzündliche Neubildung von Bindegewebe, Beitr. z. path. Anat. u. z. allg. Path. 1902, suppl. 5; Beiträge zur Histologie der eiterigen Entzündung, ibid. **38**:301, 1905.

30. Maximow, A.: Cultures of Blood Leukocytes: From Lymphocyte and Monocyte to Connective Tissue, Arch. f. exper. Zellforsch. **5**:169, 1928.

31. Bloom, W.: Mammalian Lymph in Tissue Culture: From Lymphocyte to Fibroblast, Arch. f. exper. Zellforsch. **5**:269, 1928.

of the polyblasts (exudative mononuclear cells) in inflamed tissues. This origin of the free macrophages from two apparently different sources is not hard to understand in view of the close embryogenetic relationships between the two parent cells (Maximow [32]), the great mass of work showing the histiocytic origin of the polyblasts (Ziegler,[33] Marchand, Maximow, Aschoff and Kiyono,[34] Tschaschin,[35]), the rôle of the blood lymphocytes in inflammation (Maximow [29]), and the developmental potencies of the agranulocytes in tissue cultures (Awrorow and Timofejewsky,[36] Carrel and Ebeling,[37] Maximow,[29] Lewis,[38] Lewis and Lewis,[39] Timofejewsky and Benewolenskaja,[40] Caffier,[41] Bloom [31]).

The fibroblasts all over the body were studied carefully in all of the material from these five cases. Not anywhere was there an indication of their turning into the foam cells. The fibroblasts everywhere had pale, large nuclei with scarcely visible cytoplasm, and transitional forms between them and the typical foamy histiocytes in the same tissues were never seen. This observation thus offers another negation of the theory of von Möllendorf,[42] who maintained that the fibroblasts

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32. Maximow, A.: Ueber die Entwicklung der Blut- und Bindegewebszellen beim Säugetierembryo, Folia haemat. **4**:611, 1907.

33. Ziegler, E.: Ueber die Beteiligung der Leukocyten an der Gewebsneubildung, Verhandl. d. X. Internat. Med. Cong. Berlin **2**:1, 1891.

34. Aschoff, L., and Kiyono, K.: Zur Frage der grossen Mononukleären, Folia haemat. **15**:383, 1913.

35. Tschaschin, S.: Ueber die "Ruhenden Wanderzellen" und ihre Beziehungen zu den anderen Zellformen des Bindegewebes und zu den Lymphozyten, Folia haemat. **17**:317, 1913.

36. Awrorow, P., and Timofejewsky, A.: Kultivierungsversuche von leukämischem Blute, Virchows Arch. f. path. Anat. **216**:184, 1914.

37. Carrel, A., and Ebeling, H.: Pure Cultures of Large Mononuclear Leucocytes, J. Exper. Med. **36**:365, 1922.

38. Lewis, M.: The Formation of Macrophages, Epithelioid Cells and Giant Cells from Leucocytes in Incubated Blood, Am. J. Path. **1**:91, 1925.

39. Lewis, M. R., and Lewis, W. H.: Transformation of Mononuclear Blood Cells into Macrophages, Epithelioid Cells and Giant Cells in Hanging Drop Blood Cultures from Lower Vertebrates, Contrib. Embryol., Carnegie Inst. Washington **18**: 95, 1926.

40. Timofejewsky, A., and Benewolenskaja, S.: Explantationsversuch von weissen Blutkörperchen mit Tuberkelbazillen, Arch. f. exper. Zellforsch. **2**:31, 1925.

41. Caffier, P.: Ueber die Umwandlungsfähigkeit der weissen Elemente des normalen menschlichen Blutes bei in vitro-Kultivierung, Arch. f. exper. Zellforsch. **4**:419, 1927.

42. Von Möllendorf, W., and Von Möllendorf, M.: Das Fibrocytennetz im lockeren Bindegewebe, seine Wandlungsfähigkeiten und Anteilnahme am Stoffwechsel, Ztschr. f. Zellforsch. u. mikr. Anat. **3**:503, 1926; Die örtliche Zellbildung in Gefässwanden und im Bindegewebe, München. med. Wchnschr. **73**: 135, 1927.

give rise to all of the blood and connective tissue cells. The correctness of von Möllendorf's observations was denied by Maximow,[43] Lubarsch,[44] Aschoff,[44] Büngeler,[44] Gerlach and Jores,[45] and Bloom.[46] Because of the fact that some investigators (Mallory,[47] Sabin, Doan and Cunningham,[48] Marchand, Herzog[49]) believed that the peripheral vascular endothelium could produce phagocytes, particular attention was paid, in studying the slides of Niemann-Pick's disease, to the appearance of the peripheral vascular endothelium in all of the organs and tissues. In none of the sections (see next paragraph on the liver) was there any indication of the vascular endothelium changing into large foam cells. In some places, the endothelial cells might become slightly vacuolated or show lipoid droplets with specific stains. But such cells always remained small and never rounded off and became free. These pictures recall those obtained by the intravenous injection of india ink; following such an injection, the tissues show much carbon in the endothelial cells, but these cells do not turn into free phagocytes. Even Foot,[50] who, on the basis of his work with india ink, championed the endothelial origin of the phagocytic mononuclears, has now given up this view. The nonparticipation of the peripheral vascular endothelium in the formation of the foam cells in the disease under consideration, agrees with the observations of Maximow, Lewis,[51] and Clarke and Clarke[52] on the inability of the vascular endothelium to turn into free phagocytes.

Since the so-called resting endothelial cells in the liver do turn into foam cells (Bloom, Pick), and in view of some as yet unpublished

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43. Maximow, A.: Morphology of the Mesenchymal Reactions, Arch. Path. **4**:557 (Oct.) 1927.

44. Büngeler, W.: Ueber Leukozytenbildung aus Histiozyten; with discussion by Lubarsch and Aschoff, Verhandl. d. deutsch. path. Gesellsch. **22**:243, 1927.

45. Gerlach, W., and Jores, A.: Die Herkunft der Exsudatleukozyten bei der akuten Entzündung, Virchows Arch. f. path. Anat. **267**:551, 1928.

46. Bloom, W.: Abscess Formation in B. Monocytogenes Infection, Arch. Path., to be published.

47. Mallory, F. B.: Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1923.

48. Sabin, F. R.; Doan, C. A., and Cunningham, R. S.: Discrimination of Two Types of Phagocytic Cells in Connective Tissues by the Supravital Technique, Contrib. Embryol., Carnegie Inst., Washington **16**:125, 1925.

49. Herzog, G.: Experimentelle Untersuchungen über die Einheilung von Fremdkörpern, Beitr. z. path. Anat. u. z. allg. Path. **61**:377, 1916.

50. Foot, N. C.: Studies on Endothelial Reactions: X. On the Origin of the Pulmonary "Dust Cell," Am. J. Path. **3**:413, 1927.

51. Lewis, M. R.: Origin of the Phagocytic Cells of the Lung of the Frog, Bull. Johns Hopkins Hosp. **36**:361, 1925.

52. Clarke, E. R., and Clarke, E. L.: On the Failure of Endothelial Cells, Even After Desquamation, to Be Transformed into Wandering Cells, with Observations on the Nature of Endothelium, Anat. Rec. **36**:357, 1927.

experiments on extramedullary myelopoiesis, in which I saw these cells turning into hemocytoblasts and myeloid cells, the resting endothelial cells of the liver should be considered to be of embryonic character and should be differentiated from the peripheral vascular endothelium. I have never seen a report in which the latter were demonstrated to be undergoing unquestionably a phagocytic or myeloid transformation.

It was difficult to trace the origin of the foam cells in the glomeruli. As was pointed out, not anywhere in the body was there evidence of the capillary endothelium swelling into giant foam cells, although the endothelium might contain a sprinkling of lipoid droplets. The best explanation of the foam cells in the glomeruli is that they developed as free macrophages from circulating agranulocytes which had been caught in the capillaries—as in the lungs. Moreover, if the foam cells had arisen from the endothelium of the glomeruli, one might have expected the latter to be more regularly and uniformly involved. As was described, only a few of the glomeruli contained the foam cells, and these, when present, rarely numbered more than two or three.

Essential lipoid histiocytosis is a congenital lipoid dystrophy. Data as to what the mechanism of the abnormal functioning is, have not been secured as yet. Corcan, Oberling and Dienst believe that the upset is due to malfunctioning of the hepatic cells. Little evidence has been offered in favor of this view. In my case *C*, both the Kupffer and the hepatic cells were just beginning to be affected, while the thymus, spleen and lymph nodes showed advanced changes. The tests of function carried out by Hamburger also indicated that the hepatic cells are not the primary cause of the disturbance.

In our [1] latest case, a marked vacuolization of the blood agranulocytes was noted. If this is found to be a constant feature of future cases, it should, if taken in consideration with the age of the children, the enlarged spleen and enlarged liver, the progressive cachexia and the failure to develop physically and mentally make the disease easily recognizable on purely clinical data. The chemical determination of the lipoidemia and the absence of signs of diabetes also help to make this disease distinctive.

SUMMARY AND CONCLUSIONS

The distribution and the appearance of the foam cells characteristic of Niemann-Pick's disease (essential lipoid histiocytosis) are described in some detail.

The reactive connective tissue of the body responds in this disease by mobilization, hypertrophy and accumulation of large amounts of lipoids.

The various epithelial, muscle, ganglion, and cartilage cells (all of which are functionally specifically differentiated cells) respond by lipoidal degeneration.

The connective tissue foam cells develop from the local histiocytes, as well as from the embryonic mesenchymal cells of Maximow.

The blood agranulocytes develop intravascularly into foam cells.

The fibroblasts and the peripheral capillary endothelial cells do not turn into lipoid-storing macrophages.

THE EFFECTS OF AMNIOTIC FLUID ON SEROUS SURFACES *

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Postoperative peritoneal adhesions have long been a troublesome aftermath of abdominal operations. Moreover, the tendency of the peritoneal cavity to wall off foreign bodies does not recognize the value of free drainage in certain cases, and drains are encapsulated as readily as gangrenous appendixes. Many measures have been tried to check the formation of adhesions, but none has proved satisfactory.

Kubota [1] summarized experimental results with most of the substances proposed for the prevention of adhesions, and found none of them particularly satisfactory, although one papain preparation gave fairly good results. Papain has recently been used by Ochsner [2] to prevent the re-formation, after operative lysis, of experimentally produced adhesions in the pericardium. But papain is hardly suitable for use in human beings because of the narrow zone between effectiveness and irritation, and the danger of using a powerful digestant. The use of hypertonic (20 per cent) dextrose solution was recently proposed by Buchbinder.[3] However, as the effect is sought by the production of a massive transudate, there is danger of dehydration and of distention. Moreover, the amount of sugar might well be excessive for certain patients. Morton [4] found that the presence of hypertonic dextrose solution in the peritoneal cavity did not retard absorption of phenolsulphonphthalein.

Johnson [5] reported promising results in preventing peritoneal adhesions by the use of amniotic fluid. The experiments reported in the present paper were planned to determine more fully the value of this substance.

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* Read before the American Association of Pathologists and Bacteriologists, Washington, D. C., May 1, 1928.

* From the Pathological Laboratory of the New England Deaconess and Palmer Memorial Hospitals and the Department of Pathology, Harvard Medical School.

1. Kubota, T.: Experimental Studies on the Prevention of Peritoneal Adhesions, Japan M. World **2**:226, 1922.

2. Ochsner, A.: Experimental Pericarditis, read before the American Association for Thoracic Surgery, Washington, D. C., May 1, 1928.

3. Buchbinder, J. R.: The Prevention of Peritoneal Adhesions and Encapsulation, Surg. Gynec. Obst. **45**:769, 1927.

4. Morton, C. B.: Peritoneal Absorption: Comparison between the Normal and the Inflamed Peritoneum, Am. J. M. Sc. **173**:517, 1927.

5. Johnson, H. L.: Observations on the Prevention of Postoperative Peritonitis and Abdominal Adhesions, Surg. Gynec. Obst. **45**:612, 1927.

Amniotic fluid has long been recognized as of prime importance in preventing adhesions between the fetus and the walls of the amniotic sac (Williams [6]). It is apparently a combination of transudate from the maternal blood and secretion from the fetus and amniotic epithelium. According to Uyeno,[7] it is always slightly hypotonic as compared with both fetal and maternal blood. The reaction is slightly alkaline, never acid. The specific gravity never rises above 1.010. There is always a considerable amount of coagulable albumin present, averaging 0.226 per cent of the total. A trace of globulin, and minute amounts of mucin, peptones and albumoses are found. Cholesterol is a constant component, although its amount varies; urea amounts to about 0.03 per cent, as does ammonia. Histidine, lysine and allantoin are all present in small amounts and if any arginine is to be found, the amount is still undecided. When free from meconium, the total solids make up 1.2 per cent of the fluid. Among the inorganic compounds, sodium, potassium, calcium, magnesium, phosphorus and iron are present in minute amounts.

EXPERIMENTS

Early experiments [8] were performed with whole human or bovine amniotic fluid, sterilized by Berkefeld filtration. The sterility of whole amniotic fluid was difficult to maintain. Moreover, it seemed advantageous to reduce the amount of protein present, although unfavorable reactions were not noted. Through the co-operation of the Eli Lilly Company a supply of concentrated bovine amniotic fluid, prepared by fractional alcoholic precipitation, was provided for experimental purposes.

As the prevention of postoperative adhesions was the aim, a standard operation for the production of the maximal number of peritoneal adhesions was devised in preference to the use of chemicals. In most instances, guinea-pigs were utilized, although rabbits, cats and dogs were also operated on.

Adhesions were produced as follows: The abdomen of the pig was shaved, the animal was anesthetized with ether and an incision was made in the midline through the linea alba 3 cm. in extent, beginning at a point about 1 cm. below the ensiform cartilage. A number of loops of bowel, from eight to ten, were exposed through the wound and rubbed briskly with dry gauze until there was oozing of blood from their surfaces. These loops were left exposed for a period of about two minutes. During this time, the abdominal wall was lifted up and the parietal peritoneum scraped thoroughly with a knife blade held at a right angle over an area, about 4 cm. in diameter centering at the incision. A considerable amount of red pulp on the knife bore evidence to the efficacy of this method in eroding the peritoneum. The abdomen was then sewed up with a continuous silk suture including muscle and peritoneum and with interrupted catgut sutures in the skin. Occasionally, continuous through and through sutures were used. The incision was then completely sealed over with collodion. Little effort was made to insure asepsis.

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6. Williams, J. W.: Obstetrics, ed 5, New York, D. Appleton & Company, 1924, p. 173.

7. Uyeno, D.: The Physical Properties and Chemical Composition of Human Amniotic Fluid, J. Biol. Chem. **37**:77, 1919.

Certain of these animals used as controls were then given intraperitoneal injections of 10 cc. of warm sterile sodium chloride solution. Others were not given any further treatment. The rest received 10 cc. of warm sterile amniotic fluid, which had been filtered with a Berkefeld filter, boiled or concentrated. The amniotic concentrate contained an antiseptic preparation of mercury and iodine in the dilution of 1:20,000.

The operation naturally entailed much shock, and many animals died, particularly at first, as a result of the operation. Still other animals either died or were killed by their cage mates and their entrails eaten, so that satisfactory examination was impossible.

The results of the experiments on guinea-pigs are summarized in table 1. The table includes the observations on both controls and animals

TABLE 1.—*Results of Use of Amniotic Fluid to Prevent Adhesions After "Standard Operation" on Guinea-Pigs*

Substance Used	Number of Pigs	Peritoneal Adhesions				Death from Intestinal Obstruction
		None	Slight	Moderate	Dense	
None (Control).....	24	0	0	2	19	3
0.8% NaCl (Control)..	20	0	0	4	16	0
Boiled amniotic fluid..	7	0	2	4	1	0
Filtered amniotic fluid.	10	4	2	4	0	0
Amniotic concentrate..	80	42	28	5	5	0

TABLE 2.—*Results of Injection of Amniotic Concentrate to Prevent Adhesions After Use of Tincture of Iodine*

		Peritoneal Adhesions			
	Number	None	Slight	Moderate	Dense
Controls	6	0	0	0	6
Animals treated with amniotic concentrate.....	8	6	0	1	1

treated with amniotic fluid in various forms. Only those animals in which satisfactory examinations could be made have been included.

The severity of adhesions was judged as follows: "slight," one or two delicate adhesions between loops of gut, omentum, or abdominal wall; "moderate," fairly numerous delicate adhesions between loops of gut, omentum, and abdominal wall; "dense," loops of gut and omentum matted together and closely attached to the abdominal wall.

Most of the animals in which the concentrated fluid gave little, if any, protection were treated with two of the early lots, which were inferior to those used subsequently.

Some animals were treated with amniotic concentrate in the form of a dry powder, but, owing to difficulty in the maintenance of sterility and difficulty of application, this form was discarded.

For purposes of comparison, tincture of iodine was rubbed over several loops of gut in a small series of guinea-pigs, for the purpose of producing adhesions. The results are shown in table 2.

As Warden [8] reported a few instances in which he produced convulsive seizures in female rabbits by intravenous injection of preparations of amniotic fluid of rabbits, it is important that any possibility of injurious effects should be ruled out. Earlier experiments in this laboratory showed the harmlessness of intraperitoneal injections of the fluid.[5] After passage of bovine amniotic fluid through Berkefeld V bougies, to remove any particles large enough to act as emboli, ill effects were not noted from intravenous injection of 1 to 5 cc. in both pregnant and nonpregnant guinea-pigs and rabbits.

The length of time during which amniotic concentrate persists in the peritoneal cavity eroded by the standard operation has an important

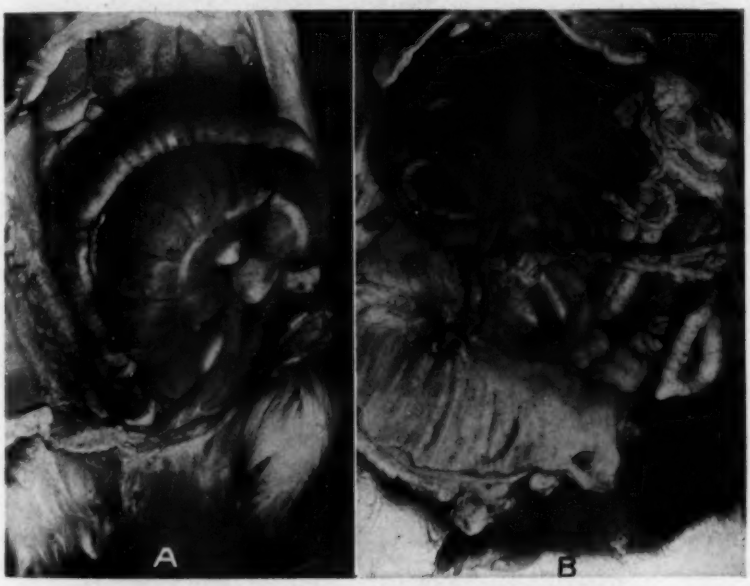

Fig. 1.—*A* reveals the peritoneal cavity of a guinea-pig eight days after an abdominal operation designed to produce as many adhesions as possible, but with the administration of 10 cc. of amniotic concentrate intraperitoneally to prevent adhesions. Adhesions were not found. *B* shows the peritoneal cavity of a control nine days after an exactly similar operation, but without the administration of the amniotic concentrate. Adhesions of the omentum to the line of incision, the colon, and the small intestine and adhesions of loops of intestine to one another and to the abdominal wall may be seen.

bearing on the effect of the fluid. To determine what effect, 10 cc. was placed in the peritoneal cavity after the standard operation had been completed and the incision sutured. At intervals of one hour for the

8. Warden, M. R.: Amniotic Fluid as a Possible Factor in the Etiology of Eclampsia, Am. J. Obst. & Gynec. **14**:292, 1927.

first eight hours, then at four hour intervals to twenty-four hours, and then at thirty-six, forty-eight and sixty hours, an animal was killed and the incision was reopened and the amount of fluid which could be sucked from the peritoneal cavity by a Luer syringe was measured. Care was taken to prevent an admixture of blood. A small amount of fluid, perhaps 0.2 cc., escaped collection due to its spreading among the viscera.

During the first four hours, the amount of fluid present drops to about one half its original volume, but after this the rate of absorption

Fig. 2.—Section from guinea-pig eighteen days after the operation, 15 cc. of filtered amniotic fluid having been injected intraperitoneally. Slight postoperative adhesions may be seen; reduced from a magnification of × 10.

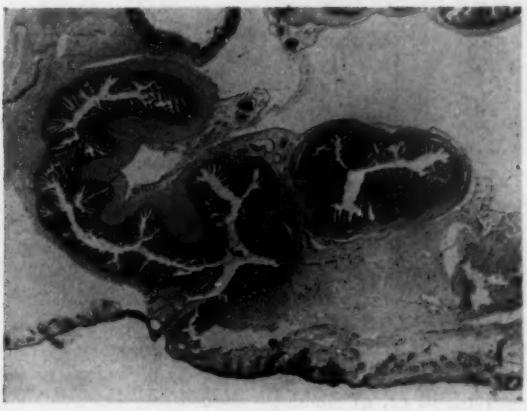

Fig. 3.—Section from control, nineteen days after an exactly similar operation. Dense adhesions; reduced from a magnification of × 10.

decreases rapidly, about 40 per cent being present at the end of the eight hours and 15 per cent at the end of twenty-four hours. Usually all the fluid was absorbed at the end of forty-eight hours, although traces have been found sixty hours after the operation.

Another important observation is that the period of oozing of the eroded peritoneum is reduced by one-half when the peritoneum is kept wet by amniotic concentrate. Oozing was considered to have ceased when dry gauze gently pressed on the eroded surface did not show a

blood stain. The average normal period of oozing was four minutes, whereas the average period of oozing of the surfaces wet with amniotic concentrate was two and a half minutes.

COMMENT

From the described experiments, it may be seen that amniotic fluid or amniotic concentrate may be used without ill effect in the peritoneal cavity of animals. Other series of experiments, now being carried out, indicate that it may be used with safety in other serous cavities.

The purpose in nature of this amniotic fluid has been variously interpreted. By some it has been considered merely a waste product from the embryo; others have seen it as a means contributing toward efficient dilatation of the os at time of labor; still others consider it as a means for preventing adhesions between the fetus and the membranes. Obstetricians have looked askance at amniotic fluid and when, in the course of cesarian operations, it has been spilled into the peritoneal cavity, every trace has been assiduously wiped out. However, it is apparent that uninfected amniotic fluid cannot do harm and may be of considerable value to the maternal organism through its action in preventing adhesions.[5]

The amniotic concentrate, prepared by fractional alcoholic precipitation, has many advantages for use over the whole amniotic fluid. The amount of protein is reduced; the concentrate is sterile, stable and easily handled.

Since amniotic fluid or concentrate prevented the formation of adhesions, the question arose as to whether or not it would interfere with healing. Wounds moistened with the fluid healed as rapidly as the wounds in the controls. Moreover, amniotic concentrate added to heparinized plasma in the proportion of 1 : 10 did not retard the growth of fibroblasts in tissue culture.

The action of the fluid in preventing the formation of postoperative adhesions is apparently due to two factors. The first is the marked reduction in the time of oozing of injured tissues. This lessens the amount of blood and fibrin on their surfaces, and hence restricts their tendency to form fibrinous adhesions which will later be organized. The second is the slowness of absorption of the fluid, permitting it to remain as a lubricant between eroded surfaces until the period when adhesions are most likely to form is past.

In view of the results of these experiments on animals, it is probably conservative to say that clinical trial of amniotic fluid will result in a reduction of postoperative adhesions in at least 60 per cent of cases and, in some instances, will completely prevent the formation of the adhe-

sions. In view of this value and the lack of danger, it seems advisable to make the use of amniotic concentrate a routine postoperative procedure in cases in which the serous cavities are involved.

SUMMARY AND CONCLUSIONS

A satisfactory method of preventing postoperative peritoneal adhesions is not in use at present. Amniotic fluid may be used with safety in the animal or human peritoneum, and it reduces the number and extent of adhesions formed. Amniotic fluid does not interfere with normal healing. It apparently acts by reducing oozing from injured surfaces, thereby lessening the amount of fibrin formed; and also, since it is but slowly absorbed, by lubricating the peritoneal cavity, until the danger of adhesions being formed is past. Amniotic fluid may be utilized to prevent the formation of adhesions in serous cavities other than the peritoneum; the amniotic concentrate is safe, more convenient, and very nearly as efficacious as whole amniotic fluid.

A FENESTRA OF THE DIAPHRAGM*

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The pathologic condition of the diaphragm described in this paper as a fenestra is sufficiently different from the usual diaphragmatic hernia to warrant a detailed presentation. The name applied was selected as an anatomic term descriptive of this pathologic entity.

REPORT OF A CASE

History.—(Case of Dr. L. B. Bell, Wesley Memorial Hospital). The previous history of the patient, who was 26 years old and married, was negative except for a normal first childbirth eighteen days before the onset of the present pain. Convalescence after childbirth had followed a normal course; the patient, after being discharged from the hospital in good condition, had resumed her household duties at home. Four days later, severe, knifelike pains began suddenly in the lower lumbar region on both sides, radiating upward between the shoulder blades. Accompanying these were intermittent cramping pains in the lower part of the abdomen, like uterine contraction pains. The intense lumbar pain continued until relieved by morphine, and never disappeared entirely. Nausea persisted more or less continuously, with occasional slight vomiting. The bowels and kidneys were regular in function.

The patient was admitted to the hospital three days after the onset of the pain. She did not appear critically ill, was in good spirits, and insisted on having her baby kept in the room with her. The temperature on entrance was 98.4 F., respiration 20, and pulse rate 76. Other observations made during the physical examination were entirely negative. Recovery from childbirth was pursuing a usual course. There was not any muscular rigidity nor any area of tenderness on the abdomen or back. Chest expansion was full and free, with normal resonance and lung borders. The breath sounds were normal. Examination of the heart did not disclose anything abnormal. Neuromuscular reflexes were normal. Results of blood and urine analyses were negative. X-ray observations did not add anything of interest.

Pain in the lumbar region was diffuse and continued severe, although its sharpness was dulled by morphine. The patient vomited only once. Shortly after midnight, she told the floor nurse that she was resting in bed easily and without pain. Fifteen minutes later, she was found on the floor of her room dead.

Pathologic Observations.—Autopsy disclosed an oval opening in the left dome of the diaphragm 11 cm. in long diameter. The long axis of this opening extended from the dome backward and laterally, and none of the structures which normally perforate the diaphragm was found within the opening. Its edges were smooth, rounded, and fibrous, without evidence of any fresh tear of tissue. Through the opening extended the ascending and descending colon and the duodenum with their mesenteric attachments, and the splenic attachment.

When the breastplate was removed, the stomach wall bulged forward under considerable pressure. The lungs and mediastinal structures were compressed

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and pushed over entirely to the right side of the chest. (A medial line drawn on the photograph shows this displacement.) The heart was not only displaced bodily, but was rotated to the right on its long axis, so that the usual posterior surface of the left ventricle presented itself anteriorly. The stomach was greatly dilated with fluid and gas. It lay with the pyloric end in the upper part of the chest and the esophageal end below, with a sharp bend of the gastro-esophageal junction. Also compressed together in the left and central portions of the chest were the greatly dilated transverse colon, the omentum, a portion of the duodenum, and the spleen and the pancreas. After puncture and drainage of the hollow viscera, all the displaced abdominal structures could be returned through the diaphragmatic fenestra to their normal sites, except the omentum. This was found firmly attached by fibrous adhesions to the anterior basal aspect of the left lung for a distance of 4 cm.

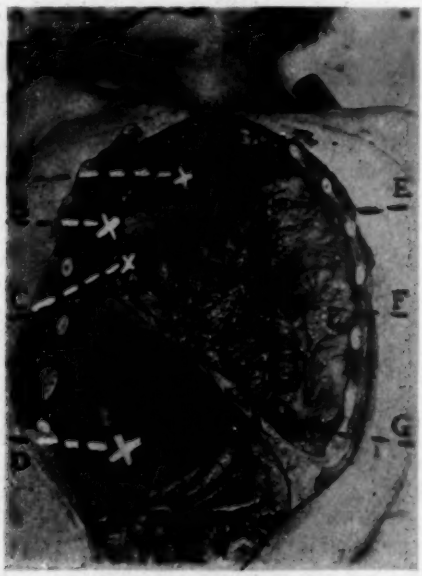

Fig. 1.—The opened chest and abdominal cavity of a patient who had died as a consequence of misplacement of internal organs through a fenestra of the diaphragm. *A* indicates the left lung, *B* the right lung, *C* the heart, *D* the liver, *E* the stomach, *F* the colon and *G* the diaphragm.

Examination of separate organs added little of significance to the observations. The breasts were hyperplastic and actively secreting. The uterus was undergoing involution at such a stage as might be found normally about a month post partum. The lungs were compressed to small size. All the thoracic and abdominal viscera were markedly congested with blood.

COMMENT

This diaphragmatic opening was considered a dilatation of a true fenestra, and not a recent rupture of a normal or a herniated diaphragm. The possibility that it might have been due to a spontaneous rupture

of a normal diaphragm is improbable. Any rupture of the diaphragm, even through an imperfectly formed area, would probably occur only at a time of exceptional stress, and the associated symptoms would present themselves promptly. There had not been anything in the previous history of the patient to suggest such an occurrence, save the recent childbirth. That the fenestra was not made at that time we have proof in the evident age of the fibrous adhesions of the omentum to the lung. That it was not a recent rupture was evidenced also by the smooth, rounded edge of firm connective tissue about the circumference of the opening. Neither was the opening due to

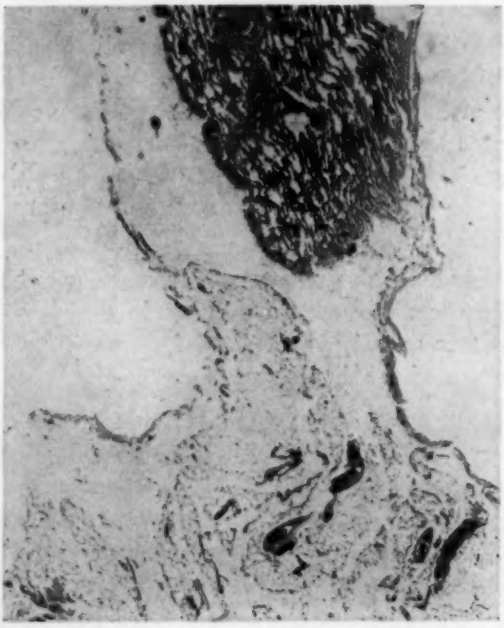

Fig. 2.—Fibrous adhesion of the omentum to the lung.

the giving way of the diaphragm around normal structures passing through it, for such structures were not found in the opening itself nor in the diaphragm at the margins of the opening.

Essentially this fenestra must be considered congenital in origin. It appeared on the left side, which is the usual site of congenital defects of the diaphragm. There are two possibilities as to its mode of development. The fenestra either existed at birth, or opened later through a congenitally weak portion of the diaphragm. It is conceivable that a small fenestra already existing at the time of the childbirth eighteen days before, was protected then by the omentum and the left lobe of the liver from protrusion of any structure through it. With the

tremendous pressure developed during expulsion of the child, the defective dome of the diaphragm may have been further weakened and thinned. This thinning allowed further dilatation of the fenestra after the patient began again doing her house work. The sudden pain in the back and the vomiting gave evidence of beginning dilatation of the fenestra, and this must have progressed beyond the limit of the holding capacity of the elastic connective tissues at the margin of the fenestra. According to the hospital record, just fifteen minutes before the patient was found dead, she was lying in bed, feeling easy so far as pain was concerned, and showing no evidence of respiratory or circulatory distress. It was supposed, from the place where she was found lying on the floor, that she had arisen to see her baby, which was in a crib in the room, and that this exertion furnished the force necessary for dilating the diaphragmatic opening to the point of giving way, as it was already on the verge of doing. The termination must have been marked by a sudden extreme dilatation of the fenestral ring and sudden passage of the abdominal viscera through the greatly widened orifice.

SUMMARY

A case is presented with sudden death from the passage of abdominal viscera into the chest through an opening in the diaphragm. Proof is furnished that an opening had existed for some time. This opening is called a fenestra of the diaphragm.

THE DIFFERENTIATION OF SCHISTOSOMA HEMATOBIUM AND SCHISTOSOMA MANSONI ACCORDING TO THE POSITION OF THE SPINE

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AND
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In a previous paper published by one of us (A. P.[1]), a case of eggs of *bilharzia* with polar spine in the wall of the appendix of a woman of the West Indies was reported. In the urine of this patient eggs with lateral spine were present. It was concluded that "the position of the spine, whether polar or lateral, does not allow the differentiation of the two kinds of *Schistosoma*."

Professor Fuelleborn of Hamburg, in a letter to one of us (A. P.), denied the possibility of judging the exact position of the spine from sections of tissue; he claimed that in sections, lateral spines might appear as polar spines, and he did not accept the pictures of polar spines as given in the article as definite proof of the presence of another kind of eggs of *Schistosoma*.

Several paraffin blocks, containing small pieces from the appendix and the cervix uteri, were sent to the Institut für Schiffs- und Tropenkrankheiten and examined by one of us (V.).

After removal of the paraffin by means of xylol, the material was brought through the alcohols into water. Digestion for twelve hours in the fresh pancreatic juice of dogs was not sufficient for isolation of the ova; after six hours in 5 per cent potassium hydroxide the eggs of *Schistosoma* became free and could be examined in the sediment. One hundred and fifty-one ova were isolated from the pieces of the appendix. Each single ovum was examined; all the ova had a lateral spine. The material from the cervix was treated in the same manner but separately; no ova could be found. In one slide from the cervix, a calcified ovum was found, but it did not show any spine, and further ova could not be found either in slides or in sediment from the cervical tissue after dissolving it in antiformin.

The conclusion was reached, therefore, that the seemingly polar spines in the wall of the appendix were in reality lateral spines corresponding to the *S. mansoni* type. To determine whether spines in eggs of *Bilharzia* are polar or ventral, observations must be made on isolated eggs and not on sections of tissue.

1. Plaut, A.: Bilharzia Infection in an Apparently Normal Appendix, Arch. Path. **1**:711 (May) 1926.

THE ALLEGED MECHANISMS OF THE LOCAL NERVES IN THE CAUSATION AND IN THE TREATMENT OF EDEMA CAUSED BY IRRITANTS*

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AND
F. L. REICHERT, M.D.
SAN FRANCISCO

The production of inflammatory reactions by irritants has been frequently attributed to reflex action of the local nerves. Modern work on the capillary responses appears to give support to this conception, although conclusive evidence of the innervation of the capillaries is still lacking. The older claims for the existence of these reflexes were based on the benefits obtained from analgesics and anesthetics, both local and systemic, used in the treatment of persons with inflammatory processes. The benefits thought of were not only the relief from pain, but a direct alleviation of all the symptoms of inflammation. Following up these suggestions, certain investigators brought forward evidence which seemed to corroborate the older views. The subject is obviously of importance in the determination of the possible fundamental cause, and the rational treatment, of inflammatory processes. Therefore, we resolved to verify, if possible, the more important experimental results on which the claims have been based, and to study a wider range of reactions to irritants, in both animals and human beings. Before proceeding to the summary of our results, it will be useful to give briefly the salient features of the literature that was mainly responsible in developing the concept here considered.

Spiess [1] is generally credited with originating the theory that inflammation is not produced by irritation or infection when the sensory and vascular reflexes are abolished. On this basis, he advocated treatment for various inflammations with local anesthetics. Bruce [2] supported Spiess's theory by claiming that a local anesthesia of the conjunctiva, or degeneration of the trigeminal nerve during eight days, in rabbits and cats, completely prevented the chemosis due to mustard oil. This prevention he attempted to explain by postulating the

* From the Departments of Pharmacology and Surgery, Stanford University School of Medicine.

1. Spiess, G.: München. med. Wchnschr. **8**:345, 1906.

2. Bruce, A. N.: Arch. f. exper. Path. u. Pharmakol. **63**:424, 1910; Quart. J. Exper. Physiol. **6**:339, 1913.

existence of an "axon reflex," similar to that previously shown by Langley [3] in the sympathetic nerves, but differing from the latter in that the pathway was from the endings of the sensory nerves up the centripetal branches of the sensory fibers and then through an axonal branch to the smaller blood vessels of the region involved. As section of the nerve trunk did not break the continuity of the reflex, the arc was obviously completed in the periphery, without cell bodies or ganglions in its pathway. Later, Bardy,[4] in verifying Bruce's work to a limited extent, added another element to this arc by postulating the presence of a ganglion cell and synapse on the motor side of the pathway. He thought the existence of such an extra neuron necessary, since the local or the systemic application of nicotine to the conjunctiva prevented the chemosis caused by mustard. Bardy was more conservative than Bruce in describing the antagonistic effects of local anesthesia, since he reported "more or less inhibition" of the irritation. Clinically, Breslauer [5] approached the problem in soldiers suffering from injuries of the nerves. From his observations, he concluded that a degeneration of the nerves diminished the reactivity of the blood vessels in the dermatitis due to mustard oil, the active vasodilatation being the part of the reaction chiefly inhibited. However, he felt that Spiess's claims were too radical and Bruce's hypothesis, doubtful. These results, therefore, already threw some doubt on the indispensability of a reflex action of the nerves in inflammatory reactions.

Our observations in both animals and man show conclusively that the mechanisms of the nerves postulated by the aforementioned investigators do not exist. We could neither confirm their results nor produce evidence along other lines to prove the necessity of the intervention of sensory nerves in inflammatory reactions. Our results may now be briefly summarized together with a critical discussion of any evidence in the literature for or against the theories which were tested.

EFFECT OF LOCAL ANESTHETICS ON CONJUNCTIVAL IRRITATION

Chemosis Caused by Mustard.—Bruce claimed that chemosis in rabbits produced by dropping mustard oil in the conjunctival sac can be prevented by first anesthetising the conjunctiva with a 10 per cent solution of alypin or cocaine. In an experiment of this kind, it is imperative that a rigorous control be had, and this should be the opposite eye, treated similarly, at the same time, except for the anesthetic solution. The eye used as control should receive the same volume of normal

3. Langley, J. N.: J. Physiol. **25**:364, 1899.

4. Bardy, H.: Skandin. Arch. f. Physiol. **32**:198, 1915.

5. Breslauer, F.: Berl. klin. Wchnschr. **55**:1073, 1918; Deutsche. Ztschr. f. Chir. **150**:50, 1919; München. med. Wchnschr. **67**:525, 1920; Zentralbl. f. Chir. **46**:723, 1919.

saline solution, since the anesthetic solution washes out the mustard oil, or mechanically diminishes its contact with the conjunctiva, and hence decreases the chemosis. Since anesthesia alone is the effect to be tested, other effects must not be produced by the anesthetic, or, if produced, they must be eliminated. Unfortunately, Bruce used his anesthetics in a strength of 10 per cent, which is much higher than necessary for complete anesthesia, and, incidentally, causes local vasoconstriction. Hirschfelder [6] showed that any measure which diminishes the peripheral flow of blood, such as a constriction of the local vessels or a fall of systemic blood pressure, results in diminution or prevention of the chemosis. This was also demonstrated by Tainter [7] for the systemic edema caused by paraphenylendiamine.

In our experiments, a 2 per cent solution of cocaine was instilled into the eyes of two rabbits, and, in addition, infiltrated into the conjunctiva and orbit of a third animal; furthermore, a 5 per cent solution of cocaine was instilled into a fourth rabbit. The opposite eyes, as controls, received at the same time volumes of saline solution equal to those of the cocaine solution. Both eyes were then treated with equal volumes of mustard oil, the operator massaging them gently to insure an even contact of the oil with the entire conjunctival surface. The anesthetized eye was tested for sensibility at frequent intervals, and complete anesthesia was maintained by applying cocaine when necessary. Under these conditions, the same degree of chemosis appeared in both eyes at about the same time. In three other rabbits, a 4 per cent solution of procaine was used in the same way as that just described for the cocaine, with negative results as to the prevention of the chemosis. The result could not be attributed to vasoconstriction, since procaine is not a vasoconstrictor.

Chemosis Caused by Ethylmorphine Hydrochloride.—As a further test of the influence of anesthesia, we tried it with another form of chemosis, namely, that produced in cats by ethylmorphine hydrochloride. After the 2 per cent solution of cocaine had been applied to the conjunctiva in three cats, application of a 10 per cent solution of ethylmorphine hydrochloride produced the same degree of chemosis in the treated eyes, despite complete anesthesia, as in the eyes used as controls. In three other cats, a 4 per cent solution of procaine was equally ineffective in modifying the amount of chemosis caused by the ethylmorphine hydrochloride.

Similar results were obtained by Stevenson and Reid,[8] who found that complete anesthesia of rabbits' eyes produced with a 10 per cent

6. Hirschfelder, A. D.: Am. J. Physiol. **70**:507, 1924.
7. Tainter, M. L.: J. Pharmacol. & Exper. Therap. **27**:207, 1926.
8. Stevenson, H. N., and Reid, M. R.: Bull. Johns Hopkins Hosp. **26**:21, 1915.

solution of alypin did not prevent chemosis following the application of mustard; and by Shimura,[9] using a 5.6 per cent solution of cocaine, and by Nakamura and Takahashi,[10] using a 2 per cent solution of cocaine and a 10 per cent solution of alypin. Groll[11] reported that inflammatory reactions in the guinea-pig occurred equally well in the anesthetized and in the nonanesthetized eyes.

Therefore, it is concluded that the chemosis caused by two different irritants (mustard oil and dionin) in three different species (rabbits, guinea-pigs and cats) was not prevented by local anesthesia and was not concerned with sensory nerves. Thus the previous postulate of an axonal reflex causing the chemosis was completely invalidated.

THE EFFECT OF COCAINE AND PROCAINE ON DERMATITIS DUE TO MUSTARD

The effect of these local anesthetics was tested by infiltrating areas of shaved abdominal skin of rabbits with a 2 per cent solution of cocaine or a 4 per cent solution of procaine and then applying mustard oil on squares of filter paper to the anesthetized regions. As controls, corresponding areas of the opposite sides were infiltrated with saline solution and treated with mustard oil in the same manner. In a series of seven experiments, differences in swelling were not observable between the nonanesthetized and the anesthetized areas. From somewhat similar experiments, Ebbecke[12] concluded that, since the vascular reactions in the anesthetized skin were maintained, an axonal reflex is not essential in the process. These observations were also in agreement with those of Tannenberg and Degener,[13] who could not demonstrate any difference between the reponses of anesthetized and of normal frogs' webs to mustard oil.

THE EFFECT OF LOCAL ANESTHETICS ON THE ENDERMIC REACTION TO HISTAMINE

Further experiments were made by us on human skin, using as test objects the wheals produced by histamine and morphine injected intracutaneously. In four persons, separate areas on the flexor surface of the forearm were infiltrated with a 1 per cent or a 2 per cent solution of cocaine, a 2 per cent solution of procaine, or a 1 per cent solution of butyn, respectively. Then histamine (0.01 to 0.02 per cent) was injected intracutaneously into the center of the anesthetized areas and also into an adjoining normal (control) area. The average diameter

9. Shimura, K.: Arch. f. path. Anat. u. Physiol. **251**:160, 1924.
10. Nakamura, M., and Takahashi, H.: Tohoku J. Exper. Med. **5**:288, 1924.
11. Groll, H.: Beitr. z. path. Anat. u. z. allg. Pathol. **70**:20, 1922.
12. Ebbecke, U.: Arch. f. d. ges. Physiol. **169**:1, 1917.
13. Tannenberg, J., and Degener, M.: Frankfurt. Ztschr. f. Path. **31**:385, 1925.

of the wheals in the areas used as controls was 13 mm.; in the cocainized areas, 14.5 mm.; in the procainized areas, 12 mm., and in the butynized areas, 12 mm. The maximal size was attained at sixteen, sixteen, nine and thirteen minutes, respectively, after the injections.

In another group of similar experiments, in which 0.01 and 0.1 per cent morphine sulphate was used to produce the wheals, we noted that the controls had an average diameter of 13 mm., with the maximal size being attained in fifteen minutes; while the cocainized areas gave wheals averaging 12.5 mm. in thirteen minutes, the procainized areas, 11 mm. in fourteen minutes, and one butynized area, 10 mm. in three minutes. From these results, it is obvious that the extent and rapidity of the dermal irritations caused by morphine and histamine were not materially altered by local anesthesia.

Our results agreed with those of Ebbecke, who also produced wheals readily in anesthetized skin. Carrier [14] concluded that the capillary reactions of stroked skin could not involve any local sensory axonal reflex, since anesthesia did not abolish them. Lewis and Grant [15] also claimed that wheals could be produced in the presence of local anesthesia, and that the increased permeability was a separate reaction to injury, independent of the reactions due to vascular tonus. The preponderance of the evidence, therefore, leads to the conclusion that local anesthesia does not prevent or modify the edema of the skin caused by irritants.

THE DERMAL RESPONSES TO MUSTARD OIL AND TO CHANGES OF TEMPERATURE AFTER DEGENERATION OF THE NERVES

It has been pointed out in an early part of the paper that Bruce and Bardy claimed prevention of the chemois produced by mustard oil through degeneration of the trigeminal nerve obtained by section, and that Breslauer gave a modicum of support to the importance of a nervous mechanism with his results in dermatitis produced by mustard in patients with degeneration of the nerves. We attempted to confirm these claims by applying mustard oil to areas of skin in which there were degenerated nerves, in rabbits and dogs, but without success. A brief summary of the results will suffice.

Responses to Mustard.—Three rabbits and five dogs were used in the first series of experiments. In the rabbits, one half of the abdominal wall was completely denervated by cutting the nerves as they emerged from the spinal muscles, sixteen days being allowed for degeneration. Mustard oil was applied simultaneously to the anesthetized area and to the corresponding normal area on the opposite side, used as a control.

14. Carrier, E. B.: Am. Physiol. **61**:528, 1922.

15. Lewis, T., and Grant, R. T.: Heart **11**:209, 1924.

In the dogs, the sciatic nerves on one side had been cut or crushed from 3 to 148 days before. They showed motor paralysis, as indicated by loss of muscular control, and anesthesia, as determined by the absence of response to painful stimuli. In addition, the dogs showed differences between the temperatures of the two limbs, the denervated side being warmer than the normal side, used as a control. These differing temperatures were of importance only so far as they indicated a disturbance in the regulatory mechanism of the peripheral vessels. This phenomenon will be described separately by one of us (F. L. R.). Mustard oil was applied to the inner surfaces of both hind legs just above the ankles. The results of the eight experiments were the same, namely: Equal degrees of edema were produced in equal time in the normal and in the denervated areas of both rabbits and dogs.

Chemosis after Denervation.—A clearcut demonstration of the lack of influence of the sympathetic nerves was obtained in three rabbits after we had excised the superior cervical sympathetic ganglion on one side. The removal of the ganglion was verified by the presence of the sensitized dilator response of the iris on the sympathectomized side, obtained by injecting minimal doses of epinephrine several days after the operation. Following eighteen days' degeneration of the nerves, mustard oil was instilled in both eyes, with the result that identical marked degrees of chemosis occurred on both sides.

Responses to Changes of Temperature.—Attempts were made to increase the differences in the circulation of the two dermal areas by exposing the animals to cold. The vessels of the skin of the normal sides responded with the usual reflex vasoconstriction, as shown by the temperature of the skin (between the toes) which fell to 22 C. (average), while that of the skin of the denervated sides averaged 32 C. Despite the marked differences in dermal circulation, as indicated by the temperatures of the skin, the degrees of edema in the normal and in the denervated areas remained equal. In these animals, the sympathetic, motor and sensory nerve fibers had all been cut and had degenerated.

It is common knowledge that a local application of cold diminishes inflammation and swelling, and that warmth increases them. Therefore, tests were made with the view of determining whether complete sensory degeneration abolished these responses to temperature in the inflammatory reaction, thus testing simultaneously for the presence and the responsiveness of any local axonal reflex in the skin.

Rabbits with unilaterally denervated abdominal walls were used. Mustard oil was applied to both the normal and the denervated sides, and both sides were kept warm with an electric heating pad arranged so that it would not exert any pressure on the areas. In several experi-

ments of this kind, large, pouchy areas of edema (1 cm. in thickness) were produced in both the normal and the denervated areas. In other words, the usual effect of heat on the inflammatory response was demonstrated both in the presence and in the absence of nerves, which have been supposed to be essential in the "axon reflex."

The effect of cooling, also, was tested. Gauze was laid over the devervated and the control areas, and water was allowed to drip on these continuously from a funnel filled with melting ice. The edema was completely prevented on both sides during a period of three hours, this result agreeing with that of the test for the effect of heat, in that the edema was affected according to the vascular response regardless of nerve supply or function.

Similar negative results, as to the theory being tested, were reported by others. For instance, Groll [11] found that the vessels of the frog's web, after section of the sciatic nerves and their degeneration, reacted normally to heat and cold, and that mustard oil produced the usual response. Similarly, Morita [16] reported definite irritation by mustard after a degeneration of the nerves in frogs and rabbits; and, also, Nakamura and Takahashi,[10] Stevenson and Reid [8] and Shimura [9] produced chemosis by irritants after a degeneration of the trigeminal nerve.

Therefore, from all this, it is concluded that a complete degeneration of the sensory and the sympathetic nerves of the skin does not prevent dermatitis being caused by mustard; that a degeneration of the sympathetic nerve fibers supplying the eye does not modify chemosis caused by mustard, and that, furthermore, the degeneration of the sensory and sympathetic nerves of the skin does not prevent the usual effects of heat and cold being manifested on the inflamed skin. Hence, it follows that the axonal reflex of Bruce, in which these nerves were supposed to be essential, is not concerned in the causation of inflammation and edema by irritants.

BARDY'S PERIPHERAL GANGLIONS AND SYNAPSES IN CHEMOSIS

In the hypothetical reflex chain under consideration, there remains the link added by Bardy,[4] namely, a peripheral synapse and ganglion which can be blocked by nicotine. The presence of this extra neuron has never been demonstrated histologically, and its existence can hardly be proved on the basis of pharmacologic data merely. Nevertheless, we repeated Bardy's experiments in connection with chemosis caused by mustard and extended them to chemosis caused by ethylmorphine hydrochloride and to dermatitis due to mustard.

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16. Morita, G.: Folia Jap. Pharm. **3**:12, 1926.

The chemosis caused by mustard was produced in three rabbits, the chemosis due to ethylmorphine hydrochloride in three cats and the dermatitis due to mustard in three rabbits. In all experiments, nicotine (1:1,000) was first infiltrated into the conjunctiva or the skin, as the case might be, the opposite sides, as controls, being injected at the same time with equal volumes of saline solution. Then the mustard or ethylmorphine hydrochloride was applied, as described in the preceding paragraphs. The net result of all these experiments was that equal degrees of edema were produced in both the normal and the nicotinized tissues. The concentration of nicotine was sufficient completely to paralyze any ganglions and synapses present. There is no reason to consider further the possibility of the existence of these structures—we could not confirm Bardy's results.

COMMENT

Our failure to confirm the results of Bruce and Bardy and to adduce new evidence in support of the alleged nervous mechanism of edema due to irritants is sufficiently plain not to require comment. It should be pointed out, however, that we have not attempted to determine whether the erythema caused by irritants is produced through an axonal reflex. As it seems well established that reactive hyperemia may be produced and spread through pathways of the nerves,[17] it follows from our experiments in which these pathways have been eliminated that such changes in vascular tonus are not indispensable to the production of inflammatory swellings. This leaves three conditions which do appear indispensable to the formation of edema when irritants are present: (1) increased permeability of the capillaries, (2) an adequate pressure in the vessels to force the fluid through the injured endothelial wall and (3) adequate fluid available for filtration. The lack of fufilment of any one of these conditions is sufficient to cause apparent inhibition or prevention of the edema and may lead to false conclusions regarding possible nervous mechanisms,—invoked, as they were, originally, largely on account of the paralytic effects of poisons (cocaine and nicotine). It would appear, then, that the irritants used, mustard oil and ethylmorphine hydrochloride, which penetrate readily, act by directly injuring the capillary endothelium.

Clinically, the results obtained by us, and by others referred to, who deny the importance of nervous mechanisms in edema, are of interest because they lead to a better understanding of the action of certain antiphlogistic agents and to rational methods of treatment for the processes of edema. We wish to suggest that the analgesic drugs, morphine, chloral, salicylates, antipyrine and cinchophen, which are

17. Lewis (footnote 15). Krogh, A.: J. Physiol. **53**:399, 1920.

employed in controlling inflammatory processes, owe their beneficial action to central depression, probably of the vasomotor mechanism, thus diminishing the intracapillary pressure, and not to interference with the local nervous mechanisms. A satisfactory method of diminishing capillary permeability directly is not at command for clinical use. For this reason, the attack on the inflammatory process must be made along other lines. The drugs of choice should be those which by constriction of the arterioles reduce the capillary pressure and diminish the peripheral flow of blood, such as epinephrine, pituitary extract, ephedrine, tyramine and phenylethanolamine. The most efficient and reliable of these in experimental edema are epinephrine and pituitary extract (Tainter[18]). The use of epinephrine appears to be well established clinically in the treatment for urticarial wheals, postoperative inflammatory swellings in the nose and throat, angioneurotic edema and the edema caused by poisoning from crab and other sea food. Pituitary extract has the advantage over epinephrine in that its action is more prolonged, but also the disadvantage that diminishing effects are produced by successive doses, if they are given at too short intervals. Although epinephrine has been tried with varying success in the exanthems caused by drugs, the rashes due to serums and the dermatitis caused by poison oak, we are not aware that pituitary extract has been so used; it would appear worthy of trial.

SUMMARY

The alleged indispensability of mechanisms of the local nerves to the production of acute edema by irritants was tested in the case of chemosis due to mustard and to ethylmorphine hydrochloride, and dermatitis caused by mustard, in rabbits, cats and dogs, and tested further in the case of wheals produced by infiltration of histamine and morphine into the human skin. The following experimentally produced conditions did not prevent or even modify the development of the edema in these cases: (1) complete local anesthesia, produced with cocaine, procaine and butyn, (2) complete degeneration of the motor, the sensory and the sympathetic nerves, and (3) infiltration with nicotine (in order to block any possibly existing peripheral ganglionic cells). Therefore, the results claimed by Bruce and Bardy were not verified, and the existence of an axon-reflex mechanism, postulated by them as being essential to the process of edema caused by irritants, is denied. These observations are in agreement with the results of other investigators.

The bearing of the observations on a more intelligent therapy in the use of the agents counteracting inflammation and edema is discussed.

18. Tainter, M. L.: J. Pharmacol. & Exper. Therap. **33**:129, 1928.

Laboratory Methods and Technical Notes

CITRIC ACID METABOLISM

The Effect of Citric Acid and Citrates on Quantitative Determinations of Acetone Body Content by the Van Slyke Method *

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In 1918, one of us (G. H. H.) noticed that quantitative determinations of the content of acetone bodies, by the method of Van Slyke, in blood in which sodium citrate was used as an anticoagulant were much higher than was suspected clinically. This study was undertaken to determine, if possible, the reason for this discrepancy. Having demonstrated, as we shall show later, that the sodium citrate was the source of error, the question arose as to the nature of the chemical reaction and whether or not it was constant and quantitative. Experiments were also done to discover whether or not citric acid, when administered in the form of lemon or orange juice, would increase the acetone content of the

Table 1.—*The Determinations of the Content of Acetone Bodies in Blood Before and After the Addition of Approximately 200 Mg. of Citric Acid Per One Hundred Cubic Centimeters*

Blood Sample	Before Mg.	After Mg.	Difference Mg.
1	5	27	22
2	3.1	30.1	27
3	...	42.7	..
4	...	40.2	..

blood as determined by this method. The effect on the determination of acetone bodies in urine was also studied. In the investigations of the blood and the urine, we had in mind a possible error in the determinations of acetone bodies in diabetic patients to whom much orange juice had been given.

Determinations were made in two samples of the same blood; one with citric acid not added, and the second with acid added in the proportion of about 200 mg. per 100 cubic centimeters of blood.

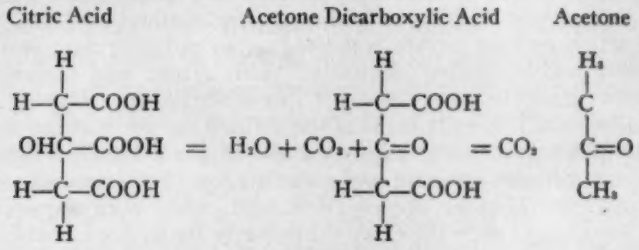
The 10 mg. of citric acid used in the second determination gave an average plus error of 35 mg.

In order to learn whether or not the reaction is a constant and quantitative one, we ran known amounts of citric acid, without blood. The results of such a series are shown in table 2.

* From the Department of Pathology of the State University of Iowa College of Medicine.

These results justify the assumption that the reaction is approximately quantitative and that 5 mg. of citric acid will produce about 20 mg. of precipitate.

It seemed probable that the citric acid was broken down to produce acetone. To check this assumption 25 mg. of citric acid was prepared as in the Van Slyke method except that the mercuric sulphate was omitted. This was then connected with a condenser and the distillate collected. Gunning's test showed the distillate to contain considerable acetone. The iodoform crystals were numerous and typical. The information obtained from the literature was rather meager, but it contained statements which justified the conclusion that citric acid when oxidized gave acetone dicarboxylic acid, acetone and carbon dioxide.[1] The following reaction was suggested:



According to this formula, approximately 80 per cent of the molecules of citric acid yield a molecule of acetone when oxidized under the conditions that obtain in Van Slyke's method for determining the content of acetone bodies.

TABLE 2.—*Increment of Precipitate for Each 5 Mg. of Citric Acid Added*

Mg. of Citric Acid	Mg. of Precipitate	Mg. of Increment
5	20	20
10	40	20
15	62	22
20	82	20
25	106	24

We next turned our attention to the effect of citric acid administered by mouth. A preliminary sample of blood was drawn and its total content of acetone bodies and its power of combining with carbon dioxide were determined. The person whose blood was used, then took 500 cc. of orange juice. Samples of blood were drawn every thirty minutes until five samples had been obtained. The content of acetone bodies and the power of combining with carbon dioxide were determined in each, with the results shown in table 3.

It appears that citric acid taken by mouth does not enter the blood stream as such in sufficient amounts to be detected by Van Slyke's

1. Von Pechmann, H.: Ueber die Acetondicarbonsäure, Ber. d. deutsch. chem. Gesellsch. **18**:2542, 1885. Richter: Organic Chemistry, Philadelphia, P. Blakiston's Son & Company, 1918, vol. 1, p. 530.

method of determining the quantity of acetone bodies in the blood. Neither does it affect the power of the blood to combine with carbon dioxide.

At the time samples of blood were taken following the ingestion of 500 cc. of orange juice, samples of urine were also obtained. The content of acetone bodies in the various samples of urine is shown in table 4. The constant figure indicates either that citric acid is not eliminated as such in the urine or that it is removed by the treatment with copper sulphate and calcium hydroxide.

TABLE 3.—*The Quantitative Determinations of Acetone and the Carbon Dioxide Combining Power of the Blood Following the Administration of 500 cc. of Orange Juice*

Blood	Acetone Bodies, Mg.	CO_2 Combining Power Val. 90
Control	0.1	78
½ hour specimen	0.4	70
1 hour specimen	0.3	72
1½ hour specimen	0.0	78
2 hour specimen	0.5	75

TABLE 4.—*The Quantitative Determinations of Acetone in Urine Following Ingestion of 500 cc. of Orange Juice*

Urine	Mg. of Precipitate
Control	1.0
½ hour specimen	0.1
1 hour specimen	0.8
1½ hour specimen	1.2
2 hour specimen	0.7

TABLE 5.—*The Quantitative Determinations of Acetone in Urine to Which Citric Acid Had Been Added Before and After Treatment with Sulphate-Hydroxide*

Amount of Acid, Mg.	Before Mg.	After Mg.
0	1.5	...
5	18.5	5.8
10	31.7	6

In order to determine whether or not citric acid is removed we ran a second series of determinations with specimens of urine to which known amounts of citric acid were added both before and after the treatment with sulphate-hydroxide.

It appears, then, that practically all of the citric acid is removed by treatment with sulphate-hydroxide. Even though citric acid were eliminated in the urine, it would not cause any appreciable error in the quantitative determination of the acetone body content by the method of Van Slyke.

CONCLUSIONS

Sodium citrate used as an anticoagulant is a source of error in quantitative determination of acetone body content in blood by the method of Van Slyke. Citric acid administered by mouth does not have any effect on either the acetone body content or the power of the blood to combine with carbon dioxide. Citric acid and its salts are not a source of error in quantitative determination of acetone body content in urine by this method.

General Review

RECENT LITERATURE ON TROPICAL SPRUE*

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RECENT CONTRIBUTIONS TO THE ETIOLOGY OF SPRUE

Sprue is defined by Manson as "an insidious, chronic, remitting, catarrhal inflammation of all of the mucous membrane of the alimentary canal, occurring particularly in Europeans who are residing or who have resided in tropical or subtropical climates." Ashford defines it as a "chronic disease characterized by a sore and beefy tongue, a gaseous bowel, a small liver, a pale, foamy, voluminous diarrhoea, wasting and anemia."

The disease was first described by Hillary in the Barbadoes in 1776. It has been variously called: aphthae tropica, diarrhea alba, white flux, Ceylon sore mouth, psilosis linguae and "sprew," the Dutch name. Kohlbrugge, in 1901, was the first to describe a monilia (albicans?) as the cause of sprue. Subsequently, Manson-Bahr and Castellani carried on important researches on sprue in the Far East. In 1915, Dr. Ashford, while working at the Institute of Tropical Medicine of Porto Rico, described a new yeastlike organism, which he named *Monilia psilosis*, as the cause of tropical sprue.

In recent years there has been a renewed and widespread interest in the subject of sprue, not only as a disease entity, but also because of the possibility of its etiology being identical with that of primary pernicious anemia. The center of interest seems to be focussed on *Monilia psilosis* of Ashford, and opinions are divided, being either for or against the organism as the cause of sprue. Confirming Ashford's work one finds the publications of Baumgartner and Smith of Clifton Springs, N. Y.; of J. M. Rogers and E. J. Wood of North Carolina; of Bastedo and Famulener of New York; of L. W. Smith of the Philippines, and of William Kraus of Memphis, Tenn. Those not in accordance with the results of Ashford are: Bovaird of New York; Hannibal and Boyd of the department of bacteriology, University of Texas; Manson-Bahr of the London School of Tropical Medicine; the staff of the Bombay Bacteriological Laboratory (India) headed by Fairley and Mackie; Warthin in Michigan, and Castellani in Ceylon.

A brief summary of two recent papers confirming Ashford's work will serve to present that side of the case.

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In a series of eleven cases, Baumgartner and Smith found *Monilia psilosis* frequently in the feces of every one of the patients. Ulcers from the mouth also gave positive cultures twice in the same patient. In blood cultures, negative results were obtained. The patients came from China, India, Korea, Porto Rico and the Philippines. The same species of monilia was isolated from the stools of patients with primary pernicious anemia in four of seventeen cases, and from the jejunum of a patient with sprue on whom autopsy was performed by them. The authors felt certain from the evidence before them that *Monilia psilosis* was more than a secondary invader and believed in the etiologic relationship of this organism to sprue.

L. W. Smith was able to confirm the work of Ashford and of Michel on complement fixation with the use of monilia antigens. He studied a small group of patients (eight in all), and agreed with Michel that there must be a toxin in *Monilia psilosis,* since guinea-pigs can be killed by the intraperitoneal injection of a saline emulsion of this organism, but not by one containing *Monilia albicans.*

From the Bombay Bacteriological Laboratory a comprehensive progress report recently appeared in the *Indian Journal of Medical Research* (1926) which did not support Ashford's work. The committee was composed of seven members who worked for eighteen months. They isolated *M. ashfordi* in half of their patients with sprue, but also in healthy persons and in patients who did not have sprue but other diseases, including various intestinal disorders. This monilia was not found with greater frequency than were the frankly nonpathogenic wild yeasts in sprue or in other conditions.

They did not find any yeast, however, which was as toxic for guinea-pigs as *M. ashfordi.* Repeated intraperitoneal injections generally produced plastic peritonitis and the death of the animals, but some animals recovered spontaneously if single doses were injected. Contrary to Ashford's results, the cultures were not exalted in virulence by passage through animals. Monkeys were also found normally to harbor *M. psilosis* in the feces.

The work of González-Martínez and of Michel was not corroborated by this committee. They were able to obtain positive complement-fixation tests only in animals in which experimental injections of *M. psilosis* were made; but not in any of eight with typical cases of sprue. Furthermore, *M. albicans* and *M. psilosis* gave common group reactions in serologic tests.

Regarding the significance of *Monilia ashfordi* in sprue, the following quotation summarizes the opinion of this committee: "Yeasts multiply more vigorously when the alkalinity of the intestine is decreased." Evidence that any yeast plays a specific rôle in the etiology of this disease or that sprue is to be regarded as a moniliasis of the digestive tract is definitely lacking. I have never found systemic involvement

of the tissues nor demonstrated *Monilia ashfordi* in pathologic lesions in the tongue or intestines at autopsy. Furthermore, the distribution of *M. ashfordi* in human excreta as reported in the Bombay series was so widespread that its presence in the feces in sprue is a matter of doubtful significance. The most that can be said regarding *M. ashfordi* is that during those phases of the disease in which its increased biologic activity can be demonstrated, it can be regarded as a contributory or secondary factor in the production of such features as abdominal distention, flatulence and acid, frothy stools, but never as the primary cause of the disease.

A bacteriologic examination of the aerobic flora in the stools in sprue was also made by the Indian commission. The organisms found were of the colon-aerogenes, salmonella and typhoid-dysentery group. The flora, therefore, resembles that of the stools in dysentery. The work is still in progress and no conclusions have been arrived at.

Of further interest in this connection are the studies on the identification of *M. psilosis* made by L. E. Hines at the McCormick Institute

TABLE 1.—*Report of the Indian Commission (Bombay)*

Clinical Diagnosis	Number of Cases	Monilia ashfordi	Atypical M. ashfordi	Monilia krusei and Nonpathogenic Yeasts
1. Sprue	49	22	14	23
2. Intestinal diseases, not sprue	35	14	8	25
3. Other diseases	14	5	7	4
4. Healthy persons	13	6	3	6
	111	47	32	58

for Infectious Diseases, Chicago (1924). This writer investigated the morphologic and bacteriologic reactions of old strains of *M. psilosis*, and concluded that they were not sufficiently characteristic for differentiation from other closely related nonpathogenic fungi. His conclusions were based on agglutination and agglutinin absorption, as well as on precipitin tests. Referring to the work of Michel and Ashford, who carried out complement-fixation tests in order to prove a pathologenic rôle for the yeasts present in the stools and lesions of the mouth of patients with sprue, Hines said: "It might be questioned whether this indicates a definite species because the controls did not include strains of other forms of pathogenic yeast such as those found in blastomycosis."

An interesting contribution by Warthin on the etiologic relationship of *M. psilosis* to pernicious anemia has just appeared. Warthin was unable to produce a hemolytic anemia by feeding old stock cultures of *M. psilosis* to guinea-pigs or by intravenous injection into rabbits. Although he produced a mycotic septicemia, there was no evidence of toxic or hemolytic action. Similarly, L. W. Smith who injected *M. psilosis* into guinea-pigs never noted a severe degree of anemia, and rarely saw lesions in the intestine, except in animals fed on a diet par-

tially deficient in vitamins. Thus it is seen that in spite of much intensive work, the exact etiology of sprue still remains a matter of dispute.

I take this opportunity to remark that the observation of *M. psilosis* in the digestive tract of many or of all patients with sprue does not prove its etiologic relationship. For, are not *B. coli* and *B. subtilis* also universally present? And can one not isolate toxins from *B. coli* and kill guinea-pigs with it by the intraperitoneal or intravenous injection of massive doses? As has been learned from the recent epidemics of influenza, Koch's postulates alone are not adequate for the establishment of an etiologic relationship between an organism and a disease. Neither are they absolutely essential. Immunologic reactions, carefully controlled, must be included. One must furthermore be on guard, as in the case of the bacillus of hog-cholera, to rule out organisms which are constantly associated with a given infection, and which may even be present in the blood stream, and yet not be the causative agent of the disease.

TABLE 2.—*Comparison of Cultural Characteristics of M. psilosis and Other Yeasts*

	M. psilosis (Ashfordi)	Normal Yeast	Yeast from Blastomycosis
Litmus milk	Alkaline	0	Alkaline
Dextrose	Acid and gas	0	Acid and gas
Saccharose	Acid and gas	0	Acid and gas
Maltose	Acid and gas	0	0
Lactose, salicin, inulin, mannite, dulcite	0	0	0

Referring now more specifically to sprue, the investigator must never lose sight of the fact that experimental moniliasis in guinea-pigs or a septicopyemia in rabbits, produced by intraperitoneal injections of massive doses of living *Monilia*, is not equivalent to sprue in man. There is still room, therefore, for a great deal of carefully controlled immunologic and bacteriologic work.

RECENT CONTRIBUTIONS TO THE PATHOLOGY OF SPRUE

Opportunities to perform autopsies in cases of sprue have been rather limited; hence, there are only a few complete reports and many quotations. Baumgartner and Thomas of Clifton Springs, N. Y., recently published their notes on one autopsy. The patient had been a teacher in the Philippines from 1905 to 1924. Before her death she had had a red cell count of less than 2,000,000; a hemoglobin content of 36 per cent, and a high color index. She also had tetany at one time, and had shown clinical evidence of degeneration of the posterior tracts of the cord, the latter symptom being suggestive of pernicious anemia. The autopsy, performed nine hours after death, showed the following: There was slight thinning of the walls of the small intestine; the liver was normal in size; the parathyroids were normal; the thoracic and

cervical cord showed only slight degenerative changes; the bone marrow was aplastic. The mesenteric nodes were enlarged; the pancreas was normal. The authors concluded that there was no characteristic pathologic change to account for the severe symptoms of the case, and that the pathologic evidence did not confirm their clinical and neurologic observations.

Laurence W. Smith found several small superficial ulcers in one case and succeeded in demonstrating mycelia in histologic sections of those lesions.

The Bombay committee reported four autopsies. They found gross intestinal ulcers in one case and enteritis of the terminal portion of the ileum in another. The pathologic changes were parallel to the general wasting of the viscera. In one case, petechial hemorrhages were seen. The lymphatics were normal. Disease of the pancreas, suprarenals or thyroid was not demonstrable. There was no involvement of the central nervous system.

The bone marrow showed hyperplasia in one case, with myeloblasts, myelocytes, normoblasts and megaloblasts, while in another case there was a complete aplasia with gelatinous, greenish-yellow marrow. In a third patient (aged 62), the picture was like that of a primary pernicious anemia. A few details of the autopsy evidence may be of interest.

Alimentary Canal.—The lesions of the tongue varied. Atrophy of the fungiform and filiform papillae was seen in two cases. Thinning of the epithelial surface and superficial invasion with yeast mycelia (unaccompanied by cellular reaction under the corium) was also observed. This was not considered of etiologic significance. The small intestine showed injection of the vessels and apparent patchy thinning of the wall in three cases; but specific atrophy in excess of the general wasting involving other viscera was not noted. Gaseous distention of the bowel was considered the cause of its production. In one case, localized enteritis of the ileum (just above the ileocecal valve) with marked congestion and injection of the vessels was found; in addition, at a higher level, petechial hemorrhages were scattered over the summit of the mucosal fold of the valvulae conniventes. In another case actual intestinal ulceration was present, a superficial healing ulcer being found in the ileum above the ileocecal valve. The liver weighed 32 ounces (908 Gm.). The kidneys, pancreas, bladder, thyroid and gallbladder were normal. The upper half of the femur was filled with hyperplastic red marrow, the lower half with yellow marrow. The parathyroids were not examined. *Monilia ashfordi* was isolated from the large intestine, but cultures from the spleen, liver, small intestine, pancreas, kidneys and bone marrow were negative.

A careful examination of another patient failed to show any evidence of lymphatic obstruction. Hence, the theory of Davenport-Jones was not supported.

Dr. R. A. Lambert, director of the School of Tropical Medicine of the University of Porto Rico, recently performed autopsies in four cases of sprue at San Juan, and it is expected that a full report on these will be published soon.

HEMATOLOGIC STUDIES IN SPRUE

Observations on the blood picture in tropical sprue have been more plentiful, yet papers are lacking which deal with the applications of the recent advances in hematology, such as the reticulocyte count, vital staining and the fragility of the red cells. There is also little data on platelets in sprue or on the size of the red blood cells. Unfortunately, too, practically all determinations of hemoglobin content have been reported on the basis of either the Tallquist or the Dare methods, both of which are subject to considerable error. There is, therefore, great need for estimations based on the recent quantitative chemical methods of Van Slyke and others. Furthermore, there does not seem to be a concensus of opinion as to the exact type of anemia that sprue represents. Perhaps it is a variable picture changing with the progress of the disease. A few summaries will now be cited.

Laurence W. Smith wrote that the blood picture in sprue was much like that of primary pernicious anemia. The red blood cells usually numbered less than 1,000,000, and the white blood cells, 3,000 or less. There was a marked relative lymphocytosis, often as high as 85 per cent, with a decrease in the polymorphonuclears. A few atypical mononuclears were seen, and rarely, basophils and eosinophils. There was an absolute decrease in the number of platelets, the average count being from 100,000 to 150,000. A slight microcytosis with no appreciable poikilocytosis and a high color index (hemoglobin content from 30 to 40 per cent) were also observed. The size of the erythrocytes was normal. Evidence of marrow activity, that is, nucleated red cells, stippling and polychromatophilia, was not observed. The number of reticulated cells was normal. Hence, the picture was like that of an idiopathic, aplastic anemia.

In a patient with tetany who came from the Philippines, Baumgartner (1927) found a hemoglobin content of 54 per cent; red blood cells, 2,200,000; color index, 1.3, and white blood cells, 4,400. Another patient, a woman (a Chinese missionary), aged 68, showed a red cell count of 1,790,000; white cells, 5,000; hemoglobin, 39 per cent; polymorphonuclears, 32 per cent; lymphocytes, 63.3 per cent, and color index —1.09.

A third patient, from China, showed a red cell count of 2,400,000; hemoglobin content, 57 per cent; color index, 1.2; white cells, 3,200; polymorphonuclears, 47 per cent, and lymphocytes, 46 per cent. All Baumgartner's patients had color indexes above 1.

Baumgartner and Thomas of Clifton Springs, N. Y., also reported a case with autopsy (1925). The patient had been a teacher in the Philippines from 1905 to 1924. Her red cell count had been less than 2,000,000, and the hemoglobin content was 36 per cent, with a color index above 1, which later changed to less than 1. A short while before her death the blood picture was as follows: red cells, 1,250,000; white cells, 1,800; hemoglobin content, 32 per cent; lymphocytes, 54 per cent, and platelets, 23,750. A few nucleated red cells and stippled cells were seen in the blood smear. The report of the Bombay Committee stated that some cases of sprue ultimately had a color index of plus 1 and ended like pernicious anemia. In a series of twenty-five consecutive cases the following averages were obtained:

Red blood cells, 3,243,490.
Hemoglobin content, 65.1 per cent.
White blood cells, 6,360.
Color index, 1.0.
Polymorphonuclears, 49.7 per cent.
Lymphocytes, 42.5 per cent.
Large mononuclears, 4.9 per cent.
Transitionals, 1.7 per cent.
Eosinophils, 1.2 per cent.

In eleven cases the color index was less than 1; in fourteen other cases it was greater than 1. The color index may vary from time to time in the same patient. The patients whose color index was greater than 1 were generally sicker than those whose index was less than 1.

Nucleated red cells and megaloblasts were rarely seen. There was usually a leukopenia with a decrease in polymorphonuclears. In the terminal picture there was a dramatic fall in hemoglobin content and number of red cells, producing a clinical condition resembling the blood crisis of pernicious anemia.

Newham, Morris and Manson-Bahr stressed the fact that anemia in sprue was secondary to a gastro-intestinal lesion and not due to a disease of the blood forming organs as in the case of pernicious anemia. In the latter disease the assimilation of food was not affected to the same degree as it was in sprue; hence, emaciation was not a feature. An increase of body fat actually occurred in some cases, and there was no wasting of the internal organs. The stools, moreover, were of normal size with a normal amount of pigment. The toxins of pernicious anemia had

definite selective action on the peripheral nerves and the central nervous system, with the production of neuritis and spinal sclerosis, features not associated with the anemia of sprue.

RECENT CHEMICAL INVESTIGATIONS OF SPRUE

Scott, of the London School of Tropical Medicine, applied the recent observations of the English physiologist Vines to the study of sprue. Vines (1921) showed that "the calcium in the plasma normally exists in two forms. Of the total (10-11 mg. per hundred cubic centimeters), some 60 per cent is readily precipitable by ammonium oxalate, whereas the rest, being bound, probably with a lipoid complex, requires nearly three times the corresponding chemical equivalent." This "bound" or combined calcium was closely concerned with the clotting of the blood. When coagulation occurred the "combined" was converted into "free" or "ionic" calcium. The parathyroids, if functioning properly, prevented the transformation of "free" into "combined" calcium.

TABLE 3.—*Calcium Content in Scott's Cases of Sprue*

Ionic Calcium	Total Calcium	Outcome of Patient
7.7	10.0	
6.8	9.9	
6.7	10.4	
6.1	9.4	Died
8.1	10.4	Mild case

In sprue, Scott found that the active or ionic calcium was deficient (from 20 to 30 per cent below normal), while the total calcium remained approximately normal. In severe cases the total was a little diminished, but never to any marked extent. The diminution was, therefore, due not to faulty absorption from the gastro-intestinal tract but to the failure of the tissues to utilize the calcium. This suggested a disturbed parathyroid function. This phenomenon was not limited to sprue, however, for it existed also in cases of malaria and in patients with severe malnutrition.

According to Scott, the percentage of ionic calcium may be utilized as a guide to the therapy of sprue and also in its differential diagnosis.

The Committee in Bombay confirmed Scott's reports of a lowering of ionic calcium in most of their fifty cases, but they did not observe any values less than 7.4 mg. per hundred cubic centimeters, and since tetany required a calcium content as low as 6 mg. per hundred cubic centimeters, their conclusion was that sprue was not caused by perverted parathyroid metabolism.

Ashford and Hernández made a study of blood calcium in sprue, and found that low total values for serum calcium were found not only in cases of sprue but in the majority of cases of serious malnutrition in Porto Rico.

Other chemical constituents of the blood were also investigated by the staff of the Bombay laboratory. The total nonprotein nitrogen, sugar and chlorides were normal. The total fat, fatty acids, cholesterol and serum albumins and globulins were low, while the fibrin was a little high.

The importance of determinations of the percentage of ionic calcium as a method of diagnosis and as an indication of the progress of the disease was confirmed. A reading of under 9.4 mg. of ionic calcium per hundred cubic centimeters was considered diagnostic of sprue. A rise in calcium in the blood was also observed in patients under parathyroid treatment. Valuable as these researches on calcium may be, they must be checked up by a careful study of the metabolism of calcium, the calcium content of the diet as well as the output in the stools being taken into consideration. As far as I am aware, metabolic studies which include determination of the basal metabolic rate in sprue have not yet been reported.

Blood tests for sugar tolerance were made in a limited number of patients by J. D. Thomson. They showed a certain degree of intolerance, most noticeable during exacerbations of the disease and associated with loss of weight. This seemed to point to a temporary inefficiency of the glycogenic function of the liver. Patients with glycosuria were given insulin by Baumgartner who found that although there was a disappearance of the sugar, no effect could be observed on the symptoms of sprue.

The bilirubin content of the serum was also determined by the workers in Bombay. High van den Bergh readings (indirect) were only exceptionally observed in sprue. This was suggestive of a secondary and not a primary type of anemia, since in pernicious anemia high indirect van den Bergh readings are the rule.

The anemia of sprue was also studied chemically by R. M. Morris of England. He found in two patients with severe anemia that the average daily output of urobilin in the urine and feces was much above the normal. From his data he calculated that the index of blood destruction was from 1.4 to 4.9 times as high as in normal cases.

The fractional test meal was used in twenty-six patients with sprue treated in Bombay. Four showed hyperchlorhydria, seven were normal, eight had hypochlorhydria and seven showed a complete absence of hydrochloric acid.

There was a definite delay in the emptying time of the stomach, but no disturbance in secretory function. "The increase of gas in the intestine brings about an increased intraduodenal tension with relative intolerance for HCl, and the pyloric sphincter becomes atonic." The authors also claimed that an examination of the fractional test meal for total chlorides, inorganic chlorides and active hydrochloric acid provided a means of differentiating sprue from true pernicious anemia.

In the latter disease, a definite achlorhydria existed owing to a secretory defect, whereas in the achlorhydria of sprue, hydrochloric acid was secreted efficiently but was neutralized by regurgitated alkaline secretions from the duodenum. (It may be added by way of explanation that the total chlorides afford an index of the secretory function of the stomach, while the inorganic chlorides are an index of hydrochloric acid which has undergone neutralization by sodium bicarbonate of the pancreatic juice).

Investigations of the duodenal contents made by an English investigator, J. D. Thomson, and independently by Van Steenis, a Dutch worker in the East Indies, as well as by Silverman in New Orleans, showed that the pancreatic enzymes were entirely normal in sprue. The bile constituents, especially the bile acid salts, were lowered. The amount of excreted urobilin was correspondingly reduced in two cases.

Ashford and Hernández, on the other hand, found an absence of lipase, a normal content of trypsin and a high acidity in the duodenal juice. The content of amylase was irregular.

The feces were reinvestigated by a German worker, Halberkann. He confirmed the well known data of Cammidge and others, that there was a marked increase in the total fats in sprue. The fats constitute from 40 to 50 per cent of the total weight as compared with a normal weight of from 6 to 8 per cent. This was due not to the failure of the fat splitting enzymes, but to an interference with the absorptive power of the upper parts of the intestine.

Manson-Bahr agreed with this view and went so far as to say that the failure to absorb fats (already digested) would explain almost the whole syndrome of sprue. He considered the ulceration of the ileum the essential lesion. Digested fats could not pass into the lacteals because of the destruction of the villi and atrophy of the mucous membrane.

The Bombay workers also examined the fat in the feces. Their results in early cases were entirely different from these in advanced cases.

In their earliest case (second day of onset) the following analysis was reported:

Solids, 13.3 per cent; total fat, 52 per cent; neutral fat, 33.6 per cent; free fatty acids, 11.6 per cent (45.2 per cent unsoaped fats); combined fatty acids, 6.6 per cent.

They concluded that in sprue it was not a failure of pancreatic function, but more probably of the activities of the enterocolon which were responsible for the high content of fat in the feces. The neutral fats were split properly, and saponification was normal, but the stools

were highly acid (p_H 4.6), irritating and gaseous. They were hurried through, and hence there was no time for absorption. This explained the large residue of undigested food found in the stools.

NUTRITIONAL STUDIES IN SPRUE

Although there are scattered remarks in the literature to the effect that sprue may be a disease of dietary insufficiency, definite or systematic laboratory researches on this subject have not yet appeared. Elders, a Dutch worker in the East Indies, believed that the diet of patients with sprue was deficient in animal proteins, amino-acids and vitamins A and B. Ashford also recently developed the conception that "sprue is a mycosis superimposed upon a state of deficiency in certain essential food elements."

DIFFERENTIAL DIAGNOSIS OF SPRUE

The disease most confused with sprue in modern medical centers is primary pernicious anemia. As stated before, a common etiology for the two diseases is being sought by many recent investigators. The Bombay committee, however, is convinced that sprue is a distinct entity. Unlike pernicious anemia, emaciation is always a concomitant feature of sprue, and the central and peripheral nervous systems are never involved. The following laboratory examinations will be found of great aid: determination of ionic calcium content of the blood, fractional test meal (with examination for total and inorganic chlorides and free hydrochloric acid), the van den Bergh test and the icterus index, as well as the cytology of the blood.

DISTRIBUTION OF SPRUE

Sprue is now known to be more widely distributed than it was originally thought to be. It has ceased to be tropical and has become cosmopolitan. Thus, from Denmark, Thaysen very recently published a report of five cases in persons who had never been outside the country. Thaysen believed that the disease was often mistaken for pancreatitis.

Many other cases of nontropical sprue were recently reported by Wood in the United States. Lambright published notes on a case which developed in Cleveland.

A series of thirteen cases of sprue, studied by Dr. David Bovaird at the Presbyterian Hospital in New York, were found to have originated anywhere from northern Korea to Ceylon and from Porto Rico to New Hampshire. The disease occurred in natives in tropical countries as well as in Americans or Europeans. The period of stay in foreign lands varied from two to thirty years and the symptoms appeared from four to twenty-four months after return from the tropics.

EPIDEMIOLOGY

The question has been raised, "Is sprue a transmissible disease?" Ashford observed in thirty-six of sixty-eight cases that other members of the family were at the same time suffering from, or had suffered from, what was presumably sprue. He was convinced that sprue was a communicable disease. But it must be recalled that all members of one family usually eat the same foods, and the possibility of sprue being a disease of dietary insufficiency must be ruled out.

The Bombay group also found multiple "infections" in the same family and among the staff of the same office. To quote from their report: "Certain bungalows and localities also have a most unsavory reputation in this respect. The vast majority of our cases were Europeans or Anglo-Indians. The outstanding predisposing factors in our series of cases were a long residence in Bombay and preceding malaria or dysentery."

RECENT ADVANCES IN THE TREATMENT OF SPRUE

Except for the introduction of a high caloric diet, rich in proteins and vitamins and low in carbohydrates and the use of selected types of fats, little new literature has appeared in this field.

Baumgartner (1927) confirmed Scott's observations that the content of ionizable calcium of the blood was low but that the total calcium was not decreased. Baumgartner recommended the use of dry parathyroid extract combined with calcium lactate. He reported a rise of calcium in the blood up to 8 mg. after the administration of 5 cc. of Collip's extract of parathyroid gland. Similar reports were made by Shepard and Fleming. Although they confirmed Scott's observations regarding the lowered content of blood in ionic calcium in sprue and observed a rise in calcium during treatment with calcium lactate and parathyroid extract, the Bombay committee has at no time been satisfied that the new treatment produced results superior to those obtained by adequate dietetic treatment, including the use of milk, meats and fruits.

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* For the sake of completeness, a few selected publications not mentioned in the text are included in this list.

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Notes and News

DOCTORATES IN MEDICAL SCIENCES CONFERRED BY AMERICAN UNIVERSITIES 1927-1928

Callie Hull and Clarence J. West, Research Information Service, National Research Council, Washington, D. C.

The following list of doctorates in Anatomy, Bacteriology, Pathology, Physiology, Physiologic Chemistry and Public Health granted during the academic year 1927-1928, has been compiled from information supplied through the courtesy of the various American universities granting the doctor's degree. Similar lists covering the years 1922-1927 were published in the ARCHIVES **1**:259 (Feb.) 1926, **3**:274 (Feb.) 1927 and **4**:808 (Nov.) 1927. These lists show the nature of the work that is carried on in the fields concerned in various universities and also persons interested in special investigations.

ANATOMY

Chicago: John Hays Bailey, "Cell Penetration by Specific Haemolysins." Rollo Clyde Baker, "The Early Development of the Ventral Part of the Neural Tube of Amblystoma." Ralph Albert Knouff, "The Origin of the Cerebral Ganglia of Rana." James Lee O'Leary, "The Form Changes in the Human Uterine Gland During the Menstrual Cycle and in Early Deciduae."

Cornell: William Mitchell Rogers, "The Development of the Pharynx and the Pharyngeal Derivatives in the White Rat (Mus Norvegicus Albinus)."

Harvard: Harold Lorraine Weatherford, "A Cytological Study of the Mammary Gland."

Michigan: William Smith Murray, "Studies of Developmental Anomalies in the Descendants of X-Rayed Mice."

New York: Pauline Kimball, "A Comparative Study of the Bas Subintestinale in the Vertebrates." Milton Overholser, "The Effect of Castration in the Albino Rat on the Size of the Parathyroid Glands and on the Susceptibility to Tatania Paratyreopriva."

Stanford: Sophie de Aberle, "Hereditary Anemia in Mice."

Wisconsin: John George Sinclair, "The Structure and Growth of Cartilage and Bone."

BACTERIOLOGY

Chicago: George W. Bachman, "A Precipitin Test in Experimental Trichiniasis." Lloyd B. Jensen, "Electrophoretic Potential and Virulence of Diphtheria Bacilli." Thurston Leown Johnson, "In Vivo Trypanolysis with Especial Reference to 'Zones of Inhibition,' Relapse Phenomena, and Immunological Specificity." John Austin Moran, "The Metabolism of Certain Anaerobic Bacteria Concerned with Food Spoilage." John Yesair, "The Action of Disinfectants on Molds."

Cincinnati: Irvin Haas Blank, "Abnormal Conditions in Animal Skins with Particular Reference to the Effect of Bacterial Activity."

Cornell: Robert Page Myers, "Germicidal Properties of Alkalies with Special Reference to the Influence of Hydroxyl Ion Concentration, Buffer Index and Osmotic Pressure." Paul Smith Prickett, "Certain Thermophilic and Thermoduric Organisms Associated with Milk." Archie Hunt Robertson, "Thermophilic and Thermoduric Micro-Organisms, with Special Reference to Species Isolated from Milk." Clifford Nicks Stark, "The Effect of Toxins on the Growth of Bacteria and the Destruction of Toxins Through Bacterial Growth."

Illinois: Luther Allen Black, "A Study of Thermophilic Bacteria from the Intestinal Tract." Glen Aven Lindsey, "Variation in Bacteria." George Ira Wallace, "Studies on the Bios Question."

Iowa State College: Lloyd Allen Burkey, "Fermentation of Cornstalks and Their Constituents: I. Studies on the Pectin Fermenting Bacteria." Gertrude Laura Sunderlin, "Studies in Home Canning: I. Some Factors Affecting the Keeping Qualities of Vegetables and Meats Canned by the Hot Water Bath Method; II. Indices of Spoilage in Home Canned Foods." Rudger Harper Walker, "Physiological Studies on the Nitrogen-Fixing Bacteria on the Genus Rhizobium." Gustav Wilster, "Clarification of Milk for American Cheddar Cheese."

Kansas: Joel G. Wahlin, "A Study of Rennin Action."

Michigan: Walter James Nungester, "Dissociation of B. Anthracis."

Minnesota: Halvor Orin Halvorson, "The Importance of Bacteria in the Transformations of Iron in Nature." Mollie Grace White, "Fermentation of Pentoses by Fusarium Lini."

New York: Mae Friedlander, "Precipitin Reaction of Antipneumococcus Serum."

Northwestern: Bertram Feuer, "Bacterial Esterases. The Influence of Lipoid, Carbohydrate, and Protein on the Production of Bacterial Esterase and a Theoretical Explanation of the Action of Bacterial Esterases."

Pennsylvania: Stanley Judson Thomas, "Doderlein's Bacillus."

St. Louis: Lloyd R. Jones, "Comparative Study of Plasma Proteins, Erythrocyte Sedimentation Rate and Precipitation of Serum Protein with Sulphate."

Wisconsin: Eldor A. Marten, "Studies in Pentose Fermentation." Myrtle Annette Shaw, "Thermophilic Bacteria in Canned Foods."

Yale: George Albert Hunt, "Biological and Chemical Studies of Lactic Acid Fermentation." Wayne Norman Plastridge, "The Influence of Gaseous Environment on Growth and Toxin Production of B. Diphtheriae, with Special Reference to Carbon Dioxide."

PATHOLOGY

California: Marion Charles Goldsworthy, "Bacterial Gummosis in the Genus Pruus." Harold E. Thomas, "Studies on Armillaria Mellea (Vahl) Quél.: Infection, Parasitism and Resistance."

Cornell: Home Symonds Cunningham, "Studies in Pathological Histology of Leaf Lesions." Frank Paden McWhorter, "The Diseases of Carica Papaya." Leva Belle Walker, "The Development of Some Basidiomycetous Fungi." James Stewart Wiant, "The Rhizoctonia Damping-Off of Conifers and Its Control by Chemical Treatment of the Soil."

Iowa State College: Makanji K. Patel, "Some Researches on the Crown Gall Pathogene, Pseudomonas Tumefaciens Sm. and Town." Gustav A. Platz, "Some Factors Influencing the Pathogenecity of Ustilago Zeae (Beckm.) Unger." Walter P. Raleigh, "Infection Studies of Diplodia Zeae and Control of Seedling Blights of Corn."

Johns Hopkins: Mary Jeannette Chapman, "A Study of Coccidiosis in an Isolated Rabbit Colony; the Clinical Symptoms, Pathology, Immunology and Attempted Therapy of the Disease." Fred Donaghy, "Studies on Immunity to a Transplantable Rat Tumor: Walker's Tape Sarcoma." Joseph Raymond Hobbs, "Studies on the Nature of the Infectious Myxoma Virus of Rabbits." Reginald Dickinson Manwell, "Relapse in Bird Malaria." Herbert Lee Ratcliffe, "The Relation of the Intensity of Trichomonad Infections in Rats on Diets of Different Protein Content to the Bacteria and Hydrogen Ion Concentration in the Cecum." Elizabeth Percy Sanders, "The Effects of Infections with Endamoeba Histolytica and Trypanosoma Equiperdum on the Blood Cells."

Minnesota: Herman Alonzo Rodenhiser, "Physiologic Specialization in Some of the Cereal Smuts." James Lewis Seal, "Studies on the Physiology of Sclerotinia Cinerea (Bon.) Wor." Cecil James Watson, "A Histologic Study of the Spleen in Various Diseases: A Study of Fresh Spleens Fixed by the Injection Method."

New York State College of Forestry: Ray R. Hirt, "The Biology of Polyporous Gilvus (Schw.) Fries."

Rutgers: Elizabeth S. Clark, "Effects of Environmental Factors and Seed Treatments on the Development of Corticium Vagum B. and C. on the Potato." John A. Small, "A Study of the Wilt Disease of Tomatoes Caused by Fusarium Lycopersici Saccardo."

Tulane: Solon Nathaniel Blackberg, "The Influence of the Lack of Vitamins on the Immunity Mechanism."

Wisconsin: Fred Anton Abegg, "Some Effects of the Waxy and the Sugary Gene in Maize on Fat Metabolism." Herbert Raleigh Angell, "Purple Blotch of Onion, Macrosporium Porri Ell." Herbert Henley Haymaker, "Tomato Wilt—Pathogenecity of Two Strains of Fusarium Lycopersici Sacc. and the Relative Toxicity of Their Excretory Products." Raymond Clyde Herrin, "Factors Affecting the Utilization of Iron in Experimental Anemia." Miguel Manresa, "A Study of the Inheritance of Resistance and Susceptibility to Infectious Abortion." Pyre Morton Simmonds, "Seedling Blight and Root Rots of Oats Caused by Fusarium Culmorum." Frederick Lovejoy Wellman, "Studies upon the Clubroot of Crucifers."

Yale: Emily Edgar McComb, "Pneumococcus Small-Flaking Agglutinins in Human Sera." Marion Banks Sherwood, "Cellular Responses and Immunity in Tuberculosis."

PHYSIOLOGY

California: Harold Francis Blum, "The Recovery of Contractility After Contraction in Cardiac Muscle."

Chicago: George Francis Cartland, "Studies in the Organic Precursors of Hemoglobin." Oscar Marvin Helmer, "A Study of Trypsin and Its Activation by Enterokinase." Onis Harrison Horrall, "Toxicity of Bile." Lemuel Clyde McGee, "Biological Activity of Testicular Extracts." Lydia Jane Roberts, "Nutrition Work with Children." Arthur H. Steinhaus, "Studies on the Physiology of Exercise." Ellen Stewart, "Alimentary Rate in the Vitamin Deficiency Diseases." Joseph Adolph Tuta, "Studies on the Oxidation-Reduction Potential of Blood." Edward Jerald Van Liere, "The Effect of Anoxemia on the Size of the Heart as Studied by the X-Ray and the Significance of the Pericardium in Acute Cardiac Dilation as Produced by Anoxemia." Russell Amos Waud, "Sudden, Transitory Reduction in the Viscosity of the Blood as a Cause of the Fall in Blood Pressure in 'Shock.'"

Colorado: Inez Ham Mattison, "The Rate of Elimination of Administered Urea as a Test of Kidney Function."

Columbia: Hattie Louise Campbell, "Growth, Reproduction and Longevity of Experimental Animals as Research Criteria in the Chemistry of Nutrition." Helen Haury Kintzi, "A Quantitative Study of the Influence of Acetate and of Phosphate on the Activity of Malt Amylase."

Ralph Kurzrok, "Biochemical Studies of Human Semen and Its Relation to Mucus of the Cervix Uteri." Harold Fisher Pierce, "Nitrocellulose Membranes of Graded Permeability."

Cornell: George Harrison Maughan, "Some Phases of Ultra-Violet Ray Treatment." Ethel Drever Simpson, "Further Experimental Studies on the Thyroid Gland."

Fordham: Alfred Lazaresco, "The Relation of Vitamin A Deficiency to Anemia." Ruth Clarke Sullivan, "The Carbohydrates in the Cerebrospinal Fluid."

Harvard: Milan Alexander Logan, "I. A Method for the Determination of Calcium in the Presence of Large Quantities of Magnesium and Phosphate; II. A Method for the Determination of Magnesium in the Presence of Large Quantities of Phosphate; III. A Study of Calcium Excretion."

Illinois: Arthur Gideon Cole, "The Proteins of Egg White: The Proteins in Egg White and Their Relationship to the Blood Proteins of the Domestic Fowl as Determined by the Precipitin Reaction."

Indiana: John Tilman Brundage, "The Isolation and Identification of Hemopoietic Substances Derived from Certain Tissues."

Iowa: Adelaide Pauline Barer, "A Critical and Experimental Study of Glycolysis." Lucea Marian Hejinian, "The Study of Growth in Infants from the Standpoint of Physical Measurements and Nitrogen Metabolism." Dempsie B. Morrison, "Biochemical Studies in Aluminum." James William Mull, "The Retention of Aluminum in the Animal Organism." Edward Muntwyler, "The Acid-Base Equilibrium of the Body: II. A Consideration of the Colorimetric Method of Estimating the Hydrogen Ion Concentration of the Blood." Robert Graham Turner, "Studies on Uric Acid: The Determination of a Previously Undetected Component of Blood and Its Relationship to Uric Acid and Thioneine." Frederick Francis Yonkman, "The Acid Intoxication of Adrenal Insufficiency in Dogs."

Iowa State College: James Boyd Allison, Jr., "Studies on the Toxicity of Hydrogen Cyanide." Fisk Gerhardt, "Physiological and Chemical Studies upon the Milkweed (Asclepias Syriaca L.)." Edward Guerrant Kelly, "Toxicity of Hydrogen Cyanide Gas on Animal Life." Clarence Clark Vernon, "Physiological Action and Chemical Constitution. New Local Anesthetics."

Johns Hopkins: Georgian Adams, "A Method for the Biological Assay of Cod Liver Oil." Ferdinand Wead Haasis, "Some Relations of Temperature and Length of Germination Period to the Percentage of Germination of Seed, Especially of Pitch Pine and Rice."

Michigan: Katharine Mabon Jarvis, "A Study of Calcium Metabolism." Genevieve Stearns, "Studies on the Intermediary Metabolism of Cystine."

Minnesota: William Earl Petersen, "A Study of Milk Fat Synthesis and Secretion." Roe Eugene Remington, "Electrical Conductivity as a Measure of the Permeability of Cells of the Beet Root." Harry Alexander Somerfield, "A Study of the Behavior of a Very Young Ovum Implanted in the Fallopian Tube." Lloyd Massena Thurston, "The Rôle of the Antiscorbutic Vitamin in the Nutrition of Calves."

Missouri: Charles Leslie Shrewsbury, "A Study of the Dietary Constituents Essential for the Nutrition of the Chick."

New York State College of Forestry: William M. Harlow, "Contributions to the Chemistry of the Plant Cell Walls."

Northwestern: Lathan Augustus Crandall, "Contributions to the Pharmacology of the Nitrites." Eric Oldberg, "I. Auto-Transplantation of the Adrenal. II. Dye Elimination by the Pancreas."

Ohio State: Russell Edmund Davis, "The Metabolism of Tributyrin." Kisaku Kitsuta, "The Storage of Calcium and Phosphorus in the Animal Body as Affected by the Vitamin A, B, B. C and D Content of the Diet." Edward Waldo Emerson Schear, "The Content of Sugar in the Blood of Mus Norwegicus Albinus When Subjected to Low Temperature."

Rutgers: Bonnie Reid Fudge, "The Relation of Initial Food Reserves of Normal and Mutilated Seeds to the Growth of Plants." O. N. Massengale, "The Study of the Effect on Bone Formation of the Ultraviolet Radiations Transmitted by a Glass Substitute."

St. Louis: Clarence J. Weber, "Studies on Carbohydrate Metabolism."

Tulane: Hazel Roberta Hafkesbring, "The Duration of Ventricular Response, Mechanically and Electrically Recorded."

Wisconsin: Wallace Parker Elmslie, "Calcium and Magnesium Relations in Animal Metabolism."

Yale: Anna Josephine Eisenman, "The Carbon Dioxide Absorption Curve of Human Blood." Martha Koehne, "The Utilization of Fatty Oils Given Parenterally." Helen Tracy Parsons, "Physiological Effects of a Dietary Rich in Protein During Pregnancy and Lactation in Rats After Removal of One Kidney." William Brandt Rose, "Vitamin B in Relation to Blood Changes, Gastric Motility and Infection."

PUBLIC HEALTH

California: Eschscholtzia Lichthardt Lucia, "A Study of Diphtheria Mortality."

Johns Hopkins: Arthur William Hedrich, "Epidemic Studies: The Monthly Variation of Measles Susceptibles in Baltimore, Maryland, from 1901 to 1928."

Massachusetts Institute of Technology: Clair Elsmere Turner, "The Experimental Development and Public Health Evaluation of an Ideal Health Education Program."

Rutgers: Anthony J. Fischer, "Factors Influencing the Decomposition of Raw Sewage Sludge."

University News, Promotions, Resignations and Appointments.—R. S. Fåhræus, known for his work on the sedimentation of red corpuscles, has been appointed professor of pathologic anatomy in the University of Upsala.

At the University of London, Robert Donaldson has been appointed to the chair of pathology at Guy's Hospital Medical School; H. D. Wright to the readership in bacteriology at University College Hospital Medical School; S. L. Baker to the readership in morbid anatomy and histology at Middlesex Hospital Medical School. The title of professor of morbid anatomy has been conferred on G. W.de P. Nicholson on account of part-time work at Guy's Hospital Medical School; and the title of professor of bacteriology has been conferred on Alexander Fleming on account of a part-time post held by him at St. Mary's Hospital Medical School.

At the Western Reserve University School of Medicine, Otto Saphir has been made assistant professor of pathology for three years and Frank R. Lyne senior instructor in clinical pathology for two years.

John F. Norton, associate professor in the department of hygiene and bacteriology of the University of Chicago, has resigned to accept the directorship of the bureau of laboratories in the department of health of Detroit.

E. M. Crookshank, emeritus professor of bacteriology at King's College, London, died recently, in his seventieth year.

Herman G. Weiskotten, professor of pathology in Syracuse University, has resigned as commissioner of health of Syracuse.

Newton Evans, professor of pathology in the College of Medical Evangelists, has been made pathologist to the County General Hospital, Los Angeles, in place of George B. Maner, resigned.

Sarah E. Branham, associate in bacteriology in the University of Rochester, New York, has been appointed associate bacteriologist at the Hygienic Laboratory in Washington, D. C.

A. M. Drenan, professor of pathology in Otago University, Dunedin, New Zealand, has been appointed professor of pathology in Queen's University, Belfast, Ireland.

James A. Doull, associate professor of epidemiology in the Johns Hopkins University, has been placed in charge of the investigation of "common colds," supported by the Chemical Foundation in New York.

The Death of Felix Marchand.—With the death of Marchand, pathologic anatomy has lost one of its most brilliant representatives. Influenced in his early scientific development by the strictly morphologic conception of diseases predominating in these days, he soon recognized the great importance of the experiment. His studies on the process of healing and regeneration will remain masterpieces of sound and critical observation for many years; the reading of these articles can be highly recommended to the young and ambitious medical scientist. His statements hold true today just as they did when they were written. During Marchand's long years as an academic teacher and head of the department of pathology at the university of Leipzig, he succeeded in establishing a great school of pathology, and many of his former pupils are now found in leading positions. Those who were lucky enough to get in closer contact with him will never forget his kind and selfless interest in their problems. He was a great scientist and an ideal friend of the students. Marchand died at the age of 82, shortly after completing his monumental contribution on inflammation in the "Handbuch der Allgemeinen Pathologie," edited by him and Krehl.

Abstracts from Current Literature

Pathologic Physiology

A Study of the Hereditary Anemia of Mice. S. B. de Aberle, Am. J. Anat. **40**:219, 1927.

Mice which are homozygous for the gene W ("dominant white") regularly suffer from a severe anemia which is invariably fatal. This hereditary anemia is first recognizable at about the sixteenth day of intra-uterine life and becomes progressively more severe. At birth all the organs of the anemic animals, except the heart, are smaller than in the controls. Experiments in transplantation and therapeutic measures lead to the conclusion that the anemia is primary, and not due to any generalized deficiency of the tissues. Detailed study of the blood of normal and anemic mice has revealed a marked deficiency in the cellular elements of the latter. The red blood cells are the first to be affected. In the anemic animal these cells are produced more slowly and disappear more rapidly than in the normal. The evidence seems to indicate that the hereditary factor is effective only in the production of red cells, all the other manifestations being secondary to an early and marked reduction in these elements. Author's Summary.

Protein Metabolism of Undernourished Children. Chi Che Wang, Jean E. Hawks and Bertha B. Hays, Am. J. Dis. Child. **35**:968, 1928.

The comparison of the nitrogen metabolism in normal and in underweight children shows the percentage of absorption to be practically the same for the two groups, while the percentage of nitrogen retained in the body as well as the retention of nitrogen per kilogram of body weight increased with the degree of undernutrition. It is suggested that the protein requirement of underweight children should be computed according to the standard, rather than to the actual, weight of the child.

Pearl Zeek.

Some Points on the Mechanism of Filtration by the Spleen. W. L. Robinson, Am. J. Path. **4**:309, 1928.

The spleen is essentially a blood filter, filtration occurring equally well in the isolated spleen as in life. The process of filtration is not dependent on the relative size of the spaces to that of the particles, nor is it dependent on the vitality of the pulp cells. The process is apparently electrophysical in nature and depends on the electrical charge of the particle in relation to that of the pulp cell. The adsorbed particles cannot be readily dislodged from the surface of the pulp cells. The spleen filters out negatively charged colloidal particles. Author's Summary.

Effects of Ovariectomy Upon Menstruation in Monkeys. E. Allen, Am. J. Physiol. **85**:471, 1928.

Ovariectomy in monkeys two weeks after the beginning of the menstrual cycle was found to cause premature menstruation, of more profuse character and longer duration than normal. In only three of the twelve animals operated on was evidence found of recent ovulation in the form of early corpora lutea. From this the writer concludes that in the absence of ovulation the endocrine function of the ovary may be continued by large or atretic follicles. The effects of the endocrine function in monkeys are the induction of growth of the genital organs to a functional state, and the manifestation of the "sexual skin phenomenon." The author interprets menstruation as an essentially degenerative process, due to decrease in, or absence of, ovarian hormone after this has been present long enough to determine a certain amount of development of the endometrium.

H. E. Eggers.

COD LIVER OIL FOR REPRODUCTION. V. E. NELSON, E. OHRBECK, R. L. JONES and M. W. TAYLOR, Am. J. Physiol. **85**:476, 1928.

A commercial brand of cod liver oil was found to furnish an adequate supply, in rats, of the fat-soluble vitamin and of vitamin E, greatly surpassing butter fat in these respects. Seventh generation rats were successfully raised with 5 per cent of this cod liver oil furnishing the only source in the diet of vitamins A, D and E. One per cent was found to supply enough vitamin E to permit ready reproduction. On the synthetic diets many of the females died at time of parturition or shortly after delivery. The fetuses and young in these cases were normal. With diets associated with failure of reproduction there was no evidence that anemia was the cause of sterility.

H. E. EGGERS.

THE INFLUENCE OF VITAMIN D DEFICIENCY ON GASEOUS EXCHANGE IN CHICKS. F. M. BALDWIN, V. E. NELSON and C. H. MCDONALD, Am. J. Physiol. **85**:482, 1928.

Rickets induced in chicks by means of a suitable diet was found to be associated with a lowering of the respiratory quotient from a normal figure of from 0.94 to 1 to one of about 0.70. The normal rate prevailed until immediately before the onset of rachitic symptoms; after this it remained low during their duration. Restoration to the diet of antirachitic vitamin by the addition of cod liver oil, or irradiation, brought about a rapid rise of respiratory quotient. During the manifestation of rachitic symptoms the temperature of the animals was slightly reduced. There was little loss of appetite.

H. E. EGGERS.

THE RÔLE OF THE ANTERIOR PITUITARY IN COMPENSATORY OVARIAN HYPERTROPHY. EARL T. ENGLE, Anat. Record **37**:275, 1928.

The more recent literature regarding compensatory hypertrophy of the ovary after unilateral or subtotal ovariectomy indicates that the factor which acts on the ovarian follicle, which is the seat of the hypertrophy, is to be sought from some extragonadal source.

That the source is the anterior lobe of the pituitary body is indicated by the cessation of cycles and of follicular formation after hypophysectomy and the complete restoration during the period of fresh gland transplants of the anterior lobe (Smith), the action of the hormone of the anterior lobe in hastening the development of the genital system and in causing superovulation, and the marked increase in size of the ovary within short periods.

Data are presented on experiments in which unilaterally ovariectomized and normal mice and rats were given the same number of daily homeotransplants of the fresh anterior lobe, and the weights of the ovaries compared with untreated unoperated, and untreated ovariectomized litter-mates. The remaining ovary from unilaterally spayed animals, which had had the same treatment as the unoperated animals, was found to weigh approximately twice as much as the combined ovarian weight of the unoperated treated animals.

Variations in ovarian weights of the treated animals occurred, due to physiologic limits of response of an organ to increased stimuli, as well as to constitutional factors. In the mouse and rat, at least, it is shown that the anterior lobe of the pituitary furnishes the stimulus for both the structural and the functional hypertrophy of the ovary observed in these experiments.

AUTHOR'S SUMMARY.

THE USE OF HISTAMINE AS A STANDARD TEST FOR DIMINISHED RESISTANCE IN SUPRARENALECTOMIZED RATS. J. MARMORSTON-GOTTESMAN and J. GOTTESMAN, J. Exper. Med. **47**:503, 1928.

Of all the drugs thus far used in testing the resistance of suprarenalectomized rats, histamine has been found to be the most satisfactory. It is a readily obtainable and comparatively stable drug, producing characteristic symptoms. Its minimal

lethal dose for a given strain of rats can be established within narrow limits, and the difference between the minimal lethal dose for normal and suprarenalestomized rats is comparatively great (1:20).

AUTHORS' SUMMARY.

THE PHYSIOLOGICAL ACTION OF HEMATOPORPHYRIN. HANS SMETANA, J. Exper. Med. **47**:593, 1928.

Feeding of hematoporphyrin to white mice had no effect on their sensitiveness to light. Staining with hematoxylin protected albino and slightly pigmented animals from sunlight even though the animals had been injected with hematoporphyrin. The pigment of the skin probably plays a physical rôle in protecting animals injected with hematoporphyrin from sunlight. In cats the injection of hematoporphyrin followed by exposure of the animal to sunlight produced changes like those in traumatic shock.

AN EXPERIMENTAL STUDY OF DIATHERMY. RONALD V. CHRISTIE, WILHELM EHRICH and CARL A. L. BINGER, J. Exper. Med. **47**:741, 1928.

An experimental pneumonia with more or less lobar distribution has been produced in dogs by the method of intrabronchial insufflation of *Bacillus Friedlanderi,* type B, and *Pneumococcus,* type I. Such dogs as showed evidences of a pulmonary lesion when photographed by x-ray were selected for measurements of lung temperature. The measurements were made by means of thermocouples before and during diathermy. The thermocouples which recorded the temperature in the consolidated lobes showed, in most instances, a more rapid rate of heating during diathermy than those in the normal lobes. The final increase in temperature in the pathologic lobes over that in the normal lobes amounted to slightly more than 1 C. When local heating occurred during diathermy it was of the order of magnitude found in a lung in which the branch of the pulmonary artery supplying it had been clamped. Histologic examination of the lungs showed the pathologic reaction to consist of an intra-alveolar exudate composed of polymorphonuclear leukocytes and desquamated alveolar epithelium. In some sections the exudate was sufficient to cause compression and emptying of the alveolar capillaries. The local heating depends on this ischemic state of the smaller vessels. Further evidence for an impaired circulation in the pneumonic lung is furnished by injection preparations; following injection of these, the uninjected area corresponded exactly with the gross pathologic lesion.

AUTHORS' SUMMARY.

PHOTOSENSITIZATION OF ANIMALS AFTER THE INGESTION OF BUCKWHEAT. CHARLES SHEARD, HAROLD D. CAYLOR and CARL SCHLOTTHAUER, J. Exper. Med. **47**:1013, 1928.

Following the ingestion of buckwheat, varicolored guinea-pigs, white swine and goats presented symptoms of photosensitization, but rabbits, dogs, white mice and rats did not. The symptoms of photosensitization were similar to those in anaphylaxis. The region of photosensitization lies between the yellow and the red ends of the spectrum. The photodynamic substance may be phyloporphyrin.

THE PRE-BERIBERI CONDITION IN JAPAN. E. C. GREY, J. Hyg. **27**:257, 1928.

A pre-beriberi condition, very prevalent in Japan, is decribed, which results from prolonged lack of proper nourishment, especially from diets too low in protein and too high in starch. Its chief manifestations are general weakness of the body, varying degrees of affection of the heart, anemia, loss of appetite and constipation. It may pass into beriberi, if the diet is not corrected. Prophylactic treatment is still more important, since the condition is slowly acquired and difficult to cure. Milk, eggs and meat are especially recommended, and natural instead of artificial foods.

PEARL ZEEK.

RELATION BETWEEN THE NATURE OF THE CARBOHYDRATE IN THE DIET AND REFECTION IN RATS. STANISLAW KAZIMIERZ KON and ELSIE WATCHHORN, J. Hyg. **27**:321, 1928.

Refection is that phenomenon in which feces of rats growing spontaneously on a diet lacking in vitamin B will, when fed to other vitamin B-free rats, cause a resumption of growth. The use of potato starch instead of rice starch in the diet of vitamin B-free rats favors spontaneous growth. Raw arrowroot starch gives similar but less effective results. The protective action of these starches is diminished by heat and alcohol.

PEARL ZEEK.

THE RELATION BETWEEN FATNESS AND STERILITY. A. S. PARKES and J. C. DRUMMOND, Brit. J. Exper. Path. **9**:63, 1928.

Rats were fed on a diet high in fat until their abdominal fat exceeded that of the controls by more than 100 per cent. In the females, the estrus cycle was not significantly disturbed nor was fertility appreciably affected. In the case of the males, however, a majority of the animals failed to breed with normal females, an effect ascribed to lethargy rather than to actual alteration of gonads. It is inferred that an excessive degree of fatness does not necessarily lead to sterility.

S. D. SIMON.

CONVULSIONS AND PARALYSIS FOLLOWING ARTIFICIAL PNEUMOTHORAX. A. BRUNERIE, Lyon méd. **140**:451, 1927.

Compressions of a left tuberculous lung were made intermittently for about eighteen months. With the last, the patient coughed at once when the needle was inserted, and the sputum was freshly blood stained. As the needle was withdrawn a marked pallor was noted, then the face became red, there was more freshly stained expectoration, and convulsions began. They were epileptiform and lasted about forty-five minutes. A slight paralysis of the left arm was noticed forty-eight hours later, but this disappeared in two more days. The attack is explained as a pleural shock and importance given fibrous adhesions of the lung. Apparently, Brunerie has never heard of the modern views which attribute these accidents to embolism of air into the cerebral arteries after it has traversed torn pulmonary vessels and the left chambers of the heart. It seems reasonable that adhesions play a part, for distention of pockets of the former pleural cavity in the attempt to compress the lung will obviously tear the lung where the inelastic fibrous bands are attached, and air or gas there under pressure enters the torn vessels and flows along with the blood to the left auricle.

E. R. LE COUNT.

TOBACCO AND BLOOD SUGAR CONCENTRATION. A. CAPONNETTO, Klin. Wchnschr. **7**:701, 1928.

Tobacco smoking causes an increase in the blood sugar of about 0.4 to 0.5 Gm. per hundred cubic centimeters. The highest value is found about two hours after the use of tobacco. Nicotine (0.001 Gm. intramuscularly) also causes an increase in the blood sugar of 0.9 to 1 Gm. per hundred cubic centimeters after from one and one-half to two hours. An effect of glycosuria was inconstant. Atropine or ergotamine injected one-half hour after the injection of nicotine suppresses the hyperglycemic effect of nicotine.

E. F. HIRSCH.

THE MUSCULAR ACTIVITY AUGMENTATION OF THE INSULIN EFFECT ON BLOOD SUGAR IN NORMAL AND DISTURBED CARBOHYDRATE METABOLISM. M. BÜRGER and H. KRAMER, Klin. Wchnschr. **7**:745, 1928.

The untreated person with diabetes has a hyperglycemia of several hours' duration following muscular activity. The effect of insulin on the blood sugar is markedly augmented by a preceding period of muscular activity. This diminu-

tion of the blood sugar by the insulin and muscular activity is not a summation effect of these two factors.

A theoretical discussion of the factors concerned with these reactions is offered by the authors.

AUTHORS' SUMMARY.

THE BLOOD SUGAR IN ARTERIAL AND VENOUS BLOOD. GEORGE ROSENOW, Klin. Wchnschr. **7**:750, 1928.

The simultaneous sampling of blood from the *A. radialis* and a vein of the arm for sugar determinations gives a better insight into carbohydrate metabolism than do estimations of venous or capillary blood only. In patients without diabetes the sugar concentration of the arterial blood is regularly greater than that of the venous blood. In diabetic patients there is no difference — the arterial and venous bloods are alike in sugar concentration.

AUTHOR'S SUMMARY.

THE INFLUENCE OF PANCREAS PREPARATIONS ON THE DISTURBED FOOD RESORPTION IN DEPANCREATIZED DOGS. M. NOTHMANN, Klin. Wchnschr. **7**:886, 1928.

The microscopic examination of feces from depancreatized dogs discloses a marked diminution of the fat and muscle fibers after the feeding of a pancreas preparation.

AUTHOR'S SUMMARY.

THE METABOLISM OF LEUKOCYTES. A. FUJITA, Klin. Wchnschr. **7**:897, 1928.

The leukocytes of the bone marrow, as well as those of the circulating blood, have the metabolism of normal embryonal cells.

AUTHOR'S SUMMARY.

PATHOGENESIS OF GASTROGENIC TETANY. H. STEINITZ, Klin. Wchnschr. **7**:932, 1928.

Tetany occurred in each of two patients with pyloric stenosis. In both, the marked depletion of chloride caused by emesia seemed to be the determining or at least the provoking factor in the manifestations of tetanus. At the same time a relative insufficiency of the parathyroid function may be a disposing factor.

AUTHOR'S SUMMARY.

THE EXCRETION OF FEMALE SEX HORMONE. M. DOHRN and W. FAURE, Klin. Wchnschr. **7**:943, 1928.

The dried feces of pregnant women has a high content of the female sex hormone.

EDWIN F. HIRSCH.

BILIRUBIN AS A HEMATOPOIETIC HORMONE. F. VERZAR and A. ZIH, Klin. Wchnschr. **7**:1031, 1928.

Bilirubin has a marked hematopoietic activity. Minimal quantities (0.5 to 3 mg. by mouth) caused a marked increase of the erythrocytes within a day, and a parallel increase of the hemoglobin content of the blood. The bilirubin or a derivative, resulting from destruction of the red blood cells by the spleen and other parts of the reticulo-endothelial system, stimulates the bone marrow to form red blood cells, and therefore is a hematopoietic hormone.

EDWIN F. HIRSCH.

CAPILLARY PRESSURE UNDER PHYSIOLOGIC AND PATHOLOGIC CONDITIONS. E. RAJKA and E. WESSELY, Ztschr. f. d. ges. exper. Med. **57**:171, 1927.

The measurements were made with a Török-Rayka-Wessely capillary tonometer; the back of the hand, or of a finger, was used. With the instrument employed the normal capillary pressure was found to be low, from 0.5 to 5 cm. of water. There was considerable seasonal variation: the pressure was lowest during

the summer months (from 0.5 to 1.7), it rose during the spring (from 1.05 to 2.5), increased further during the fall (from 2 to 3.5) and was greatest during the winter (from 3.5 to 5 cm. of water). Ultraviolet rays caused a decrease of capillary pressure in normal as well as in cyanotic or inflamed regions. The seasonal variations are believed to be due to the action of ultraviolet rays. Age had little or no effect. Increase of intrathoracic pressure, through inspiration, caused an increase of capillary pressure. Within certain limits, capillary pressure was found to be independent of arterial pressure. Contraction of capillaries (could, epinephrine hydrochloride) increased the pressure; compression of veins had a similar effect. The capillary pressure was increased in inflamed and hyperemic regions; in general the increase was parallel to the severity of the inflammatory reaction.

B. Lucké.

Importance of the Reticulo-Endothelial System with Respect to Erythrocytes. Stefan Weiss, Ztschr. f. d. ges. exper. Med. **57**:404, 1927.

It has been reported that removal of the spleen brings about changes in the number and fragility of erythrocytes. Since the spleen contains a considerable proportion of the reticulo-endothelial system, Weiss investigated the question whether the effect on erythrocytes of splenectomy is due to the sudden removal of large numbers of reticulo-endothelial cells, or to other factors. For the purpose of this study the reticulo-endothelial system of five rabbits was "blocked" by intravenous injections of saccharated ferric oxide over a period of several weeks. The number of the erythrocytes and their fragility were determined before, during and after the course of injections. While complete "blockage" of the reticulo-endothelial system cannot be accomplished, Weiss believes it possible to depress its activities. He gives a table which shows a gradual decrease (from 12 to 23 per cent) in the number of erythrocytes. The fragility of the red blood cells increased (that is, their resistance to hypotonic solution decreased) in every animal (from 0.48 to 0.56 per cent sodium chloride; from 0.50 to 0.58 per cent sodium chloride). These results are not in accord with observations after splenectomy, for this operation is usually followed by an increase in erythrocytes, and a decrease (or no change) in their fragility.

In two patients with polycythemia, who were given repeated intravenous injections of saccharated ferric oxide, the number of erythrocytes declined from 8,200,000 to 5,200,000 (during a period of two months) in one patient; while in the other patient changes were not noted (but the injections were continued for two weeks only).

B. Lucké.

Reflex Slowing of the Cardiac Rhythm and Its Dependence on Electrolytes in Blood. A. J. Smirnow and P. D. Olefirenko, Ztschr. f. d. ges. exper. Med. **57**:559, 1927.

In cats and dogs the cord was transected immediately below the medulla, care being taken not to injure the latter; artificial respiration was used. The vagus, cervical sympathetic, superior laryngeal and intraorbital nerves were transected on one side. Calcium chloride and magnesium chloride were injected intravenously. The blood pressure was recorded in the usual manner. The proximal ends of the cut ends of one or another of the nerves were stimulated. It was found that intravenous injections of calcium chloride increased the reflex slowing of the cardiac rhythm, while those of magnesium chloride diminished or abolished the reflex slowing of the cardiac rhythm; an injection of calcium chloride restored the irritability. The reflex irritability of the vagus was more pronounced in cats than in dogs.

B. Lucké.

The Influence of Removal of the Thyroid on the Glands in the Skin. M. Minowada, Acta derm. **11**:231, 1928.

In cats functional disturbances of the sweat glands become apparent in about fourteen days after removal of the thyroid. Later anatomic changes develop in the sweat glands and other glands of the skin.

Pathologic Anatomy

POSTMORTEM FINDINGS IN ACUTE JELLY-FISH POISONING WITH SUDDEN DEATH IN STATUS LYMPHATICUS. H. W. WADE, Am. J. Trop. Med. **8**:233, 1928.

An autopsy is reported of a case of acute poisoning from a jelly-fish with sudden death. The patient was a well developed young man of status lymphaticus type, with slight residual evidences of epilepsy. Besides the local injury at the site of contact with the tentacles of the jelly-fish, there was pulmonary edema, engorgement of the right side of the heart, congested viscera and evidence of injury to the parenchyma of the kidney.

PEARL ZEEK.

TUMOR-LIKE STRUCTURES IN THE LUNGS OF GUINEA-PIGS ARTIFICIALLY EXPOSED TO SILICA DUST. HENRY STUART WILLIS and PAUL BRUTSAERT, Am. Rev. Tuberc. **17**:268, 1928.

In seven of eighty guinea-pigs, subjected to the prolonged inhalation of atmospheres of silicon-carbide dust, tumor-like structures of the bronchial mucosa were found. The lungs exhibited few evidences of a fibrous reaction, such as would be expected from long continued irritation of the dust. Similar tumor-like structures were never observed in the lungs of other guinea-pigs.

H. J. CORPER.

A PATHOLOGIC STUDY OF X-RAY SHADOWS OF CHRONIC PULMONARY TUBERCULOSIS. PHILIP H. PIERSON, Am. Rev. Tuberc. **17**:331, 1928.

Horizontal sectioning through the thorax offers a method of studying carefully all the disease in a given section of the roentgenogram, including that which is otherwise obscured by the heart and diaphragm, and of correlating it with the physical signs. It also offers a means of visualizing parts (chambers, great vessels, etc.) of the heart in the roentgenogram. The method is completely described and illustrated.

H. J. CORPER.

SPONGIOBLASTOMA MULTIFORME OF THE BRAIN. LOYAL DAVIS, Ann. Surg. **77**: 8, 1928.

This is a report of a spongioblastoma multiforme of the brain with metastatic nodules in the left lung and in the tissue over the scapula and right arm. The case is reported because of the relative infrequency of distant metastases.

N. ENZER.

HEMOCIDAL PROPERTIES OF THE BLOOD SERUM IN PERNICIOUS ANEMIA. O. H. HORRALL and T. E. BUCHMAN, Arch. Int. Med. **41**:417, 1928.

A critical review is given of the literature on destruction of erythrocytes in pernicious anemia with consideration of evidence of increased red blood cell phagocytosis or other forms of destruction by various organs or tissues of the body; of isolation of toxins against red blood cells and of alteration of fragility of red blood cells.

The authors made sodium chloride dilution fragility tests on the red cells in patients with anemia; also serum dilutions with water, similar in type to the fragility test mentioned, were tested with red cells. Results with serum dilutions were expressed, according to freezing point depression, as percentage of sodium chloride. The following combinations were used: abnormal serum plus normal cells; abnormal serum plus abnormal cells; abnormal cells plus normal serum; normal cells pus normal serum.

It is concluded that hemolytic substances are present in the blood serum in pernicious anemia with relapse, hemolytic jaundice and septic anemia. The red cells are abnormal in polycythemia, aplastic anemia and secondary anemia.

H. R. FISHBACK.

CHRONIC SPLENOMEGALY. W. C. MACCARTY, Arch. Int. Med. **41**:536, 1928.

MacCarty analyzes his studies of 320 spleens from patients with splenic anemia, hepatic cirrhosis, hemolytic icterus, pernicious anemia, myelogenous leukemia, Gaucher's disease, hemorrhagic purpura, and a group with splenomegaly but an indefinite clinical picture. These groups are considered separately as to age and sex of patients, size of spleen and histologic features, such as congestion, pigmentation, thickening or hyalinization of blood vessels, and the presence of various cells. The nomenclature of the cells is that previously suggested by the author. Results are expressed as percentage of cases of the group in which the various elements are present.

Diagnosis of the clinical entity is not satisfactorily made by examination of the spleen alone. Exception may be made in extreme cases of myelogenous leukemia, Gaucher's disease and types of splenomegaly belonging to either splenic anemia or hepatic cirrhosis.

H. R. FISHBACK.

COARCTATION OF THE AORTA. L. M. BLACKFORD, Arch. Int. Med. **41**:702, 1928.

Narrowing of the aorta near the insertion of the ligamentum arteriosum has been described by many observers with various theories concerning the cause. It may be due to anomalous development of the fourth branchial arch.

Coarctation in young children has been reported in 127 necropsies. The infantile type, with narrowing proximal to the ductus arteriosus, is rapidly fatal, while the adult type may not be a factor in causing death.

Coarctation in older persons has been reported in 196 necropsies. In more than 70 per cent of these cases death was from cardiovascular causes, such as gradual or sudden cardiac failure, spontaneous rupture of the aorta, cerebral hemorrhage and subacute bacterial endocarditis. In many cases, coarctation was associated with other developmental cardiovascular defects.

Coarctation has been diagnosed clinically in seventy-eight cases, and proved by autopsy in twenty-one of these. The diagnosis may be suggested by marked difference of blood pressure in the arm and leg; pulsating superficial arteries of the chest; an unusually high pulse pressure, or the presence of a loud systolic murmur in the left interscapular space.

H. R. FISHBACK.

CHONDROMA OF THE SPINE ASSOCIATED WITH A TRANSVERSE MYELITIS. J. FELSEN, Arch. Int. Med. **41**:736, 1928.

A case is presented of chondroma of the first and second dorsal vertebrae, with slowly developing transverse myelitis, and death after two years from the onset of symptoms.

H. R. FISHBACK.

PRIMARY TUMORS OF THE SUPRARENAL CAPSULE WITH ESPECIAL REFERENCE TO SUPRARENAL VIRILISM. A. H. CROSBIE and L. W. SMITH, J. Urol. **19**: 241, 1928.

A woman, aged 38, ill with pain in the left side, chills and fever and pus in the urine, had the voice, pubes and hips of a man, coarse hair on the chest and abdomen, a coarse, dry, red skin, a blood pressure of 130 systolic and 90 diastolic, and a clitoris 4 cm. long. Her menses had started at the age of 13; they were regular but stopped when she was 29 years of age. She had had two miscarriages at three and five months, respectively; her sexual desire was normal, but intercourse was difficult because of the large clitoris. A typical suprarenal adenoma of cortical cell origin, 17 by 12 by 10 cm. and degenerated centrally was removed at operation. Six weeks later, the patient had a menstrual period, and her skin was softer and less red. In addition to the report of this case, the embryology, physiology and pathologic histology of the suprarenal gland was considered.

Two general functions of the suprarenal gland are to act as a detoxifying agent and to assist in cholesterin metabolism. Two special functions are the production of epinephrine by the medulla aided by the catalytic action of the cortex and the regulation in some degree of growth and development, particularly of the sex organs, by the cortex. Tumors of the suprarenal medulla are usually malignant and commonly of the neuroblastoma sympathicum type; less frequently ganglioneuromas consisting of adult chromaffin cells are associated with increased blood pressure. The tumors of the cortex are less regularly malignant and range from accessory bits of cortical tissue in the suprarenal capsule to hypernephromas. The various types of virilism associated with disease of the suprarenal gland are classified by Gallais as: (1) congenital true suprarenal pseudohermaphroditism; (2) prepuberty virilism—the commonest form; (3) adult group—associated with menstrual disorders, and (4) obstetric form which occurs at the menopause.

GEORGE RUKSTINAT.

RETROPERITONEAL CYST: APPARENTLY OF INTESTINAL ORIGIN. A. A. SALVIN, M. J. & Rec. **127**:256, 1928.

A painless, fluctuating mass grew gradually in the left side of the abdomen. This mass proved to be a cyst, the wall of which was lined by a single layer of high cylindric epithelial cells similar to those in the lining of the small intestine. The cyst is regarded as enterogenous in nature.

MENSTRUAL FISTULAE. M. BALLIN, Surg. Gynec. Obst. **46**:525, 1928.

Under the term menstrual fistula, Ballin considers two varieties, namely, those due to direct communication with the uterine or tubal mucosa and those due to postoperative enclosures of endometrial tissue in scars. He cites Monat's five varieties of endometriosis: (1) primary endometriosis—direct invasion of myometrium by endometrial cells; (2) peritoneal or implantation endometriosis; (3) transplantation endometriosis in abdominal scars; (4) metastatic endometriosis extraperitoneal along the round ligament and in hernia sacs (comparable to metastasis from carcinoma); (5) developmental misplaced endometrical tissue.

Both of Ballins' varieties of menstrual fistulae seem particularly likely to develop in the face of operations for ventral fixation when deep sutures predispose to transplants. Histologically, such tumor transplants contain the typical gland structures made up of endothelial cells forming blood containing cysts. Clinically, they are both painful and often hard to eradicate.

EDWIN JORDAN.

AINHUM, A BAND SCLERODERMA. HUGH W. ACTON, Indian J. M. Research **15**: 1085, 1928.

Ainhum is not a disease but a lesion caused by a band scleroderma giving rise to a spontaneous amputation of the little toe, and more rarely affecting other digits like the fourth toe and occasionally the little finger. There is a deficiency of the calcium content of the blood, indicating a deficiency of the parathyroid function and a partial association with hypothyroidism.

AUTHOR'S SUMMARY.

DIVERTICULUM OF HUMAN HEART. M. R. DRENNAN and G. T. VAN DER VIJVER, South Africa M. J. **2**:58, 1928.

A case of sudden death from the rupture of a ventricular diverticulum of the heart is reported in a child, aged 4 years. The condition found seemed to indicate that the vortex of superficial muscle fibers at the left apex may be a weak spot at which diverticulum may form. In the case described there was no valvular or other cardiac lesion that could have caused chronic strain on the muscle of the heart.

SERUM CULTURES OF YOUNG AND ADULT MAMMALIAN TISSUES AND THEIR RELATION TO GROWTH PROCESSES IN VIVO. M. J. A. DES LIGNERIS, Publications of The South African Institute for Medical Research **3**:257, 1928.

Serum, autogenous or homologous, is a good medium for the cultures of mammalian tissues, especially if diluted with one third of its volume of 50 per cent Tyrode solution. The cells that grow are those of the histiocyte-trephocyte type.

A PRIMARY MALIGNANT CHORDOMA OF LUSCHKA'S POUCH. R. ARGAUD and D. CLERMONT, Ann. d'anat. path. **5**:140, 1928.

A report of a case of a primary malignant chordoma originating in the nasopharyngeal wall in the vicinity of the Luschka's pouch is given with a review of five cases from the literature.

B. M. FRIED.

TISSUE CULTURE IN VITRO: A GENERAL REVIEW. E. FAURÉ-FREMIET and B. EPHRUSSI, Ann. d'anat. path. **5**:157, 1928.

A detailed scholarly review on the subject of tissue cultures is given.

B. M. FRIED.

ECHINOCOCCUS EMBOLISM. A. CONSTANT and H. G. HUIDOBRO, Bol. de la Soc. de cirug. de Chile **5**:293, 1927.

An instance is described of embolism in the right iliac artery caused by a hydatid cyst in a girl, aged 17 years.

THE EFFECT OF ETHER NARCOSIS ON THE FUNCTION OF THE LIVER. KURT BOSHAMER, Klin. Wchnschr. **7**:445, 1928.

Every ether narcosis causes a mild damage to the liver, which becomes marked if a liver injury already exists. Regeneration of the liver in these patients seems to be less. A latent liver injury occurs with chronic cholecystitis, alcoholism, chronic pancreatitis, carcinoma and chronic suppurations.

AUTHOR'S SUMMARY.

HUMAN KELOIDOSIS. H. SCHRIDDE, Klin. Wchnschr. **7**:582, 1928.

Schridde describes keloidosis in several different types of cases; on the skin following acne, vaccination, furuncles and wounds and also in the lungs and lymph nodes of stone cutters. He believes that keloidosis may arise as a result of physical and chemical irritation and that there is also a born tendency to keloid formation. The silicic acid present in the stone dust is the chemical agent which he thinks is responsible for the condition in the stone cutters. Histologically, keloids are much alike. They are essentially fibrous tissue cells with relatively few capillaries. Hyaline bands spread between the fibrous tissue strands and become more and more numerous as the age of the keloid increases.

THE HISTOBIOLOGY OF MYELOGENOUS LEUKEMIA WITH ROENTGEN-RAY THERAPY. F. FREUND, Klin. Wchnschr. **7**:977, 1928.

Others have expressed the opinion that leukemic cells are derived from certain cells of the local tissues. Freund believes this view correct and has observed the transitions of cells of the areolar tissues, the fat cells and those of the entire vessel wall, into leukemic cells. Under the influence of roentgen rays the leukemic cells reverted to connective tissue cells and connective tissue.

AUTHOR'S SUMMARY.

HEMOCHROMATOSIS AND LEAD. MAX ROSENBERG, Klin. Wchnschr. **7**:5050, 1928.

Hemochromatosis was observed in a man who had been intensively exposed to lead for sixteen years. Clinically, the symptoms of hemochromatosis were noticed with the first attack of lead poisoning. Lead as a causal agent deserves consideration with hemochromatosis (just as copper has been given an etiologic rôle by Mallory).

E. F. HIRSCH.

CHRONIC EXPERIMENTS WITH VITAL DYES ON RABBITS. MARGARETE HESSE, Ztschr. f. d. ges. exper. Med. **59**:15, 1928.

For the purpose of studying the effect of prolonged administration of vital dyes, lithium carmine, trypan blue and india ink were injected intravenously daily for a period of from several months to a year. The animals did not show any ill effects. In general, the dyes were stored within the same types of cells which in more acute experiments take up vital dyes and particulate matter. Storage was most prominent in the reticulo-endothelial cells of the liver, spleen, lymph nodes and bone marrow, and less marked in the endothelial cells of the suprarenals and the histiocytes of the connective tissues. All degrees of storage were found, from slight ingestion to seemingly complete saturation. In the liver, spleen and lymph nodes were groups of multinucleated giant cells, engorged with dye particles; these syncytia were interpreted as derivatives of the reticulo-endothelial cells. The experiments show the apparently unlimited ability of the reticulo-endothelial system to store particulate matter. New cells are constantly formed. It is therefore impossible to block this system completely.

B. LUCKÉ.

HISTOCHEMIC DEMONSTRATION OF MERCURY. ERWIN CHRISTELLER and R. SAMMARTINO, Ztschr. f. d. ges. exper. Med. **60**:11, 1928.

Several methods are given. The most successful are modifications of two methods devised by Almkvist, and an adaption of a method used by Christeller for histochemical demonstration of gold. Tissues are fixed for two or three days in either one of the following three solutions: (1) Hydrogen sulphide water containing 4 per cent nitric acid. (2) Picric acid—nitric acid saturated with hydrogen sulphide (add 3 cc. of 25 per cent nitric acid to 100 cc. of saturated aqueous solution of picric acid. After precipitation has occurred shake frequently and allow to stand for twenty-four hours; shake again, filter and saturate with hydrogen sulphide). (3) Distilled water 25 Gm., zinc chloride ($SnCl_2$) 2 Gm., nitric acid 1 Gm. After fixation, the tissues are embedded in paraffin. Mercury appears in the form of black granules or droplets. Care must be used to differentiate such deposits from formaldehyde precipitates or from pigments. By using these methods the authors were able to demonstrate mercury in various organs of animals experimentally poisoned with different mercurials. The results were also successful in human material obtained post mortem from patients recently treated with mercury, and from suicides. The main deposits of mercury were found at the site of local injection (in the animals), the kidneys, liver and lungs, large intestines and spleen. In small amounts mercury was found in other organs. The cells of the reticulo-endothelial system appeared to store mercury. In a postscript the authors make reference to a publication by C. Lombardo (*Arch. di farmacol. sper. sc.*, 1908, vol. 7, pt. 8-9) in which a similar method is given.

B. LUCKÉ.

EXPERIMENTAL TRAUMATIC HYDROCEPHALUS. M. HASHIGUCHI, Arb. a. d. neurol. Inst. a. d. Wien. Univ. **29**:109, 1927.

Because ventriculography has demonstrated hydrocephalus in patients suffering from traumatic neuroses, an attempt was made to produce hydrocephalus in dogs. After the animals were deeply anesthetized, the heads were tapped with a lead hammer one hundred times on each side. In spite of care, slight fractures resulted

in two of the seventeen dogs. The outcome is much more of a contribution to cerebral concussion and contussion than to hydrocephalus, for edema followed by necrosis of nerve cells and an atrophy of the brain evident grossly were found. Some proliferation of subependymal neuroglia was also noted. This would probably help to produce hydrocephalus by narrowing the passage outward of the fluid in the ventricles. Hashiguchi suggests that the time of observation, three months, was not long enough to allow hydrocephalus to develop. A slight hydrocephalus was also found in one of four dogs used as controls.

E. R. Le Count.

Pathologic Chemistry

The Biochemical Changes in the Blood Following Radium Therapy. H. B. Matthews and V. P. Mazzola, Am. J. Obst. & Gynec. **16**:97, 1928.

The report is based on the result in 100 cases in which chemical analyses of the blood were made before and after radium treatment. The blood urea was elevated slightly in both benign and malignant conditions after the use of radium. No definite relation could be established between increased blood urea and the reaction. The carbon dioxide combining power of the blood was not affected, and the results did not indicate that any renal impairment followed irradiation.

A. J. Kobak.

Measurements of the p_H of Normal, Fetal and Neoplastic Tissues by Means of the Glass Electrode. H. Millet, J. Biol. Chem. **78**:281, 1928.

The p_H characteristic of normal tissue is approximately 7.1; of nonmalignant neoplastic tissue, 6.9; and of malignant neoplastic tissue, 6.8. The p_H of fetal tissue increases with the age of the fetus. It reaches the more alkaline value of normal tissue within about twelve hours of birth.

Arthur Locke.

A Colorimetric Method for the Determination of Free Tryptophan in Blood. C. A. Cary, J. Biol. Chem. **78**:377, 1928. The Free Tryptophan in Cow Blood and Its Utilization in Milk Secretion. C. A. Cary and E. B. Meigs, Ibid., p. 399.

A method is described for the approximation of the nonprotein tryptophan content of blood and blood plasma.

A comparison of the free tryptophan content of blood plasma drawn from the jugular and mammary veins of lactating cows indicates that as much as 17 per cent of the available supply of this important amino-acid may be abstracted from the blood during its circulation through the mammary tissues.

Arthur Locke.

Changes in the Oxygen Capacity of the Blood Pigment of Rabbits Following Partial Hepatectomy. B. B. Stimson and M. C. Hrubetz, J. Biol. Chem. **78**:413, 1928.

From three to four hours following removal of three fourths of the tissue of the liver in rabbits, discrepancies become apparent between the total blood pigment concentration and the capacity of the blood to transport oxygen. These discrepancies persist for only a few hours. They may be explained by the assumption of a transient appearance, in the blood, of substances resembling hemoglobin but lacking the latter's capacity to combine reversibly with oxygen. Similar substances have been observed to appear in the blood following splenectomy. No suggestion is made concerning their nature, origin or significance other than the statement that they appear to be different spectrophotometrically from either oxyhemoglobin or methemoglobin.

Arthur Locke.

THE ESTIMATION OF ALUMINUM IN ANIMAL TISSUES. V. C. MYERS, J. W. MULL and D. B. MORRISON, J. Biol. Chem. **78**:595, 1928. THE INFLUENCE OF THE ADMINISTRATION OF ALUMINUM UPON THE ALUMINUM CONTENT OF THE TISSUES OF THE DOG. Ibid., p. 615. THE ALUMINUM CONTENT OF HUMAN AUTOPSY TISSUE. Ibid., p. 625.

The aluminum content of animal tissues may be estimated, within the range of 0.1 and 5 mg. per kilogram of body weight, with an accuracy of 90 per cent. Aluminum is normally present in dog tissues in amounts varying from 0.7 to 1.5 mg. per kilogram of body weight. These amounts hold relatively constant, except in the tissue of the liver, in which the aluminum concentration may become temporarily increased by as much as 100 per cent during maintenance on an aluminum-rich diet. The aluminum concentrations found in the heart and brain of a human being, after death, appear to be much higher than those found in the corresponding tissues of the rat and dog.

ARTHUR LOCKE.

THE EFFECT OF SODIUM CITRATE, ACETATE AND LACTATE ON THE ULTRAFILTRABILITY OF SERUM CALCIUM. D. H. SHELLING and H. L. MASLOW, J. Biol. Chem. **78**:661, 1928.

The fatal result which attends the intravenous injection of 30 per cent citrate solution into rabbits is preceded by a reduction in the nondiffusible serum calcium concentration from the normal value of from 53 to 55 per cent to a value approaching 0 per cent.

ARTHUR LOCKE.

THE SEQUENCE OF CHANGES IN THE BLOOD AND BILE FOLLOWING THE INTRAVENOUS INJECTION OF BILE OR ITS CONSTITUENTS. C. H. GREENE and A. M. SNELL, J. Biol. Chem. **78**:691, 1928.

Glycocholic and taurocholic acids disappear rapidly from the blood stream following intravenous administration to dogs. The acids may be recovered quantitatively from the bile within two hours of the injection. Their presence in the blood produces an increase in the volume of bile excreted, but no essential change in its composition. Injected bilirubin is excreted into the bile more slowly, producing a gradual increase in the biliary pigment concentration, but no corresponding increase in the rate of flow.

ARTHUR LOCKE.

BILIRUBIN AND THE VAN DEN BERGH REACTION. C. E. NEWMAN, Brit. J. Exper. Path. **9**:112, 1928.

Experimental observations on the chemistry of the van den Bergh reaction are noted, which tend to reconcile divergent hypotheses recently put forward by various workers on the subject. The observations are as follows: 1. Azobilirubin is an indicator with a double color change. 2. Biphasic serums can be analyzed into, and synthesized from, pigments giving prompt and delayed reactions. 3. Pure bilirubin is not the same substance as the pigment giving the delayed reaction in nature. 4. There are four chemically distinct bilirubins: (*a*) free acid bilirubin, made by acidifying *B*, *C* or *D*, gives the delayed reaction with a blue color, and is easily converted into salts or oxidized; (*b*) delayed reaction bilirubin, in normal and hemolytic jaundiced serums, can be made from *A* or *C* by standing in serum in an oxygen-free atmosphere and gives a delayed reaction with a rose color; (*c*) sodium hydrogen bilirubinate, in obstructive jaundiced serums and in bile, gives a prompt reaction with a rose color and is converted to *A* by acids and to *B* by standing in serum, and (*d*) disodium bilirubinate does not exist in nature but can be made from *A*, *B* or *C* by excess of soda.

PEARL ZEEK.

THE MINERAL CONTENT AND THE TISSUE REACTION OF TUBERCULOUS LUNGS. R. SCHAEFER, Beitr. z. Klin. d. Tuberk. **69**:86, 1928.

Diseased lungs, particularly tuberculous ones, contain more water and less ash than normal lungs. The amount of potassium and sodium chloride is decreased

beyond the degree of the increase in water. In some lungs with chronic tuberculosis, the calcium content may be definitely increased. The total content of the lung of a normal guinea-pig in acid and alkaline ions is definitely smaller than that in the liver, kidney and muscle. The free alkalinity is three and one-half times as large as the free acidity, while in other organs the two values are approximately equal. In tuberculous lungs of guinea-pigs, the discrepancy between the two values is still greater. Ash content, sodium chloride content and actual reaction in the sputum vary exceedingly from day to day, but these variations are not dependent on mere concentration or dilution. The sodium chloride curve always moves in the same direction as the ash curve, but its actual variations are definitely higher, which indicates a selective secretion. The actual reaction of the sputum changes greatly without any noticeable regularity. Gerson's diet (poor in sodium chloride) has no noticeable influence on the chemistry of the sputum.

MAX PINNER.

UROCHROMOGEN. E. SCHUNTERMANN and F. K. HOFFMAN, Beitr. z. Klin. d. Tuberk. **69**:438, 1928.

Urochrome was prepared in pure substance. It is an oxidation product of urochromogen. It has an aromatic nucleus and sulphur within the molecule. Based on this fact, urochromogen can be estimated quantitatively as urochrome.

MAX PINNER.

THE REACTION OF PUS IN CHRONIC AND ACUTE ABSCESSES. B. KOLDAJEW and B. KUTZENOK, Beitr. z. Klin. d. Tuberk. **69**:472, 1928.

The p_H of pus in closed tuberculous abscesses varies between 6.8 and 7.2. Acute purulations of nontuberculous origin have a p_H of from 5.8 to 6.8. The occurrence of a mixed infection in a tuberculous abscess decreases the p_H.

MAX PINNER.

Microbiology and Parasitology

RHEUMATIC FEVER AND STREPTOCOCCUS CARDIO-ARTHRITIDIS. JAMES CRAIG SMALL, Am. J. M. Sc. **175**:638 and 650, 1928.

Streptococcus cardio-arthritidis, isolated from the blood and throats of patients with rheumatic fever, is described as a new species, showing definite cultural and serologic characteristics. Its specificity is shown by its association with the disease, by its ability to produce experimental lesions in animals, and by the presence of its agglutinins and opsonins in patients' serum. The local and systemic reactions to the antiserum, vaccine and soluble antigen are described, together with the report of the use of these products in the treatment of various acute and chronic manifestations of the disease.

S. D. SIMON.

SOME GENERAL ASPECTS OF PATHOLOGICAL CONDITIONS CAUSED BY FILTRABLE VIRUSES. T. M. RIVERS, Am. J. Path. **4**:91, 1928.

There are diseases caused by certain peculiar incitants, viruses, that produce in their hosts pathologic changes not entirely unlike those found in other diseases, yet sufficiently different from them in regard to phenomena related to proliferation and degeneration to warrant placing such agents in a group by themselves. If proliferative phenomena predominate, pathologic conditions such as warts, molluscum contagiosum and tumors result. If destructive or retrograde changes prevail, diseases such as varicella, vesicular stomatitis and lysis of bacteria are the consequence. Cells affected by many of the diseases in the group exhibit intracellular changes sufficiently characteristic to be spoken of as inclusion bodies. These structures are probably closely related to the proliferative and denegerative phenomena induced in cells by the action of viruses. The views expressed in the

present paper concerning the pathologic conditions observed in virus disease are consistent with living or with lifeless incitants multiplying either intracellularly or extracellularly. The pathologic changes, however, as well as other phenomena, emphasize the fact that in virus diseases an intimate type of parasitism exists. A useful bibliography accompanies this paper.

DENGUE FEVER: TRANSMISSION BY AEDES AEGYPTI. PAUL A. SCHULE, Am. J. Trop. Med. **8**:203, 1928.

An experiment was performed to determine the length of time which must elapse before *Aedes aegypti*, having bitten a patient with dengue fever, can transmit the disease to another susceptible human being. The disease was transmitted when the interval was eight, ten or more days, but not at intervals of two, three, four and six days.

PEARL ZEEK.

SOME OBSERVATIONS ON THE INCIDENCE OF MALARIA AMONG AFRICAN CHILDREN. HENRY HANSON, Am. J. Trop. Med. **8**:249, 1928.

The incidence of malaria in the Ibadan area of Nigeria, West Africa, was studied. A very high percentage of children had malarial parasites in the blood, while adults commonly presented negative smears. It is believed that repeated infections in childhood lead to an acquired immunity which becomes so great in adult life that the individuals are no longer infected.

PEARL ZEEK.

ELECTROCARDIOGRAPHIC STUDIES IN PULMONARY TUBERCULOSIS. SALING SIMON and FELIX BAUM, Am. Rev. Tuberc. **17**:159, 1928.

The electrocardiogram seems to be of value in pulmonary tuberculosis. The low potential sometimes found in lead I or III points to the presence of drop heart or cor pendulum. The average value in height of the T wave would indicate that a weakened cardiac musculature exists in tuberculous patients, a condition long suspected by clinicians but not proved except on autopsy. Collapse treatment does not always influence the electrocardiographic picture. A right ventricular predominance (right axis deviation) was present in 10 per cent of the cases studied. A left ventricular predominance was also present in 10 per cent of these cases. An inverted T wave in two leads was seen only in cases in which the prognosis was doubtful or unfavorable.

H. J. CORPER.

THE FATE OF HUMAN AND AVIAN TUBERCLE BACILLI IN THE LIVER OF BIRDS. JOHN C. ROGERS, Am. Rev. Tuberc. **17**:192, 1928.

The intravenous injection of tubercle bacilli into pigeons results in rapid and extensive localization of the bacilli in the liver. This localization is accomplished by the phagocytic action of the vascular endothelium constituting the intima of the venous sinusoids. The great bulk of the tubercle bacilli thus localized are digested within the phagocytic cells rapidly, there being no evidence that tubercle bacilli are more resistant to digestion than are other bacteria, notably the pneumococcus. Except in those instances in which enormous numbers of bacilli are injected, morphologic changes other than those displayed by the hemophages do not appear in the liver. When large doses of virulent avian tubercle bacilli are injected, permanent tissue changes occur which include the formation of tubercles and giant cells.

H. J. CORPER.

THE WHITE BLOOD CELLS IN HUMAN TUBERCULOSIS AS STUDIED BY THE SUPRAVITAL TECHNIC. R. S. CUNNINGHAM and EDNA H. TOMPKINS, Am. Rev. Tuberc. **17**:204, 1928.

The white blood cells studied by the supravital technic may be used in diagnosis and prognosis of tuberculosis in man. The monocytes in the blood are practically

always increased in number and stimulated in phagocytic ability in tuberculosis. These changes parallel the grade of clinical severity and are index of anatomic extent of lesion and dissemination. The lymphocytes in the blood are an index of resistance and parallel increased resistance. The ratio of monocytes to lymphocytes is a useful mathematical expression for the extent of invasion compared to that of resistance. It is practically always elevated in tuberculosis and parallels increased clinical severity. Tuberculosis of the lymph nodes tends to give higher monocytic and lower lymphocytic values than are indicated by the grade of clinical severity. Children tend to show higher monocytic and lower lymphocytic values than are indicated by the grade of clinical severity. Tuberculous exudates give a characteristic cell reaction, with high lymphocytic and monocytic counts. The monocytes tend to the epithelioid type of cell. The strength of reaction to tuberculin does not bear any relationship to the changes in the blood in tuberculosis.

H. J. Corper.

The Pulmonary Effect of Over Three Years' Exposure of Guinea-Pigs to Silica Dust. Henry Stuart Willis, Am. Rev. Tuberc. **17**:253, 1928.

Guinea-pigs exposed for over three years to the inhalation of heavy atmospheres of a so-called hard dust, made up mostly of silicon carbide (98 per cent), revealed unexpectedly slight effects on the pulmonary tissues in view of the character of the dust and the prolonged contact with it. Guinea-pigs thus exposed to silica dust did not appear to be unduly predisposed to pulmonary tuberculosis on artificial infection.

H. J. Corper.

Experimental Abscess of the Lung in the Dog. S. J. Crowe and J. E. Scarff, Arch. Surg. **16**:176, 1928.

After 3,500 tonsillectomies were studied in which special precautions were taken to prevent aspiration of material, and in which not one postoperative abscess of the lung developed, Crowe and Scarff concluded that postoperative pulmonary abscess is due to aspiration rather than to the liberation of infected emboli. Their experiments on dogs gave the following results: Cotton pledgets saturated with infected scrapings from pyorrheal cavities were inserted into the main bronchus. They were thus able to produce abscess of the lung in eight animals. These were discrete and not associated with a generalized pneumonitis. In two dogs, similar abscesses resulted from inserting similar pledgets into the frontal sinus. A frontal sinusitis developed, giving rise to a purulent nasal discharge. But in fifty dogs in which the pledgets inserted were saturated with cultures of organisms, either abscess did not develop, or a diffuse inflammation resulted.

N. Enzer.

Maduromycosis: Fourth Case Reported in the United States. Harold L. Thompson and Kano Skeda, Arch. Surg. **16**:764, 1928.

The fourth case of this disease encountered in the United States was found in a negro, aged 39, who presented a swelling in the right foot. There had been intermittent drainage from the swelling, which had discharged black granular material and blood. The swelling was about half the size of an egg, with small openings on the surface, from which a dark blood-stained fluid could be expressed. Microscopic examination of the discharge revealed numerous large segmented mycelia. The tumor was excised and presented a gristly, cartilaginous structure, with irregular islands of dark brown granules. On the microscopic section, communicating abscesses containing brown granules were found, surrounded by granulation and fibrous tissue, and numerous mycelia. The prominent cells were plasma cells and large foam cells, with occasional multinucleated, foreign body giant cells. Gammel of Western Reserve studied the mycelium of this case and defines it as a new species.

N. Enzer.

INCREASING HUMAN INCIDENCE OF BROAD TAPEWORM INFESTATION IN GREAT LAKES REGION. ALDRED SCOTT WARTHIN, J. A. M. A. **90**:2080, 1928.

"The story of the development of fish tapeworm infestation in the United States is an interesting tale adorned with several morals. Its various chapters may be summarized as follows: cases of imported worms in Baltic region immigrants; disposal of ova-containing sewage into our lakes; plerocercoids in native fish; native-born hosts of the adult fish tapeworm; continuous spread of infested fish; commercial use of such fish, and, finally, occurrence of human infestation in cities far removed from endemic areas."

LEPROSY BACILLI IN "NEGATIVE LEPERS" AND IN PLACENTA AND UMBILICAL CORD. E. V. PINEDA, Philippine Islands M. A. J. **8**:65 and 67, 1928.

After apparent cure from leprosy with freedom from bacilli as determined by ordinary methods of examination, bacilli may be demonstrable in the deeper organs (nerves, lymph nodes and testes). This may be the case for as long as two years after apparent cure. Leprosy bacilli were found in the placenta and also in the blood of the cord, but there were no changes in those structures attributable to leprosy. When leprosy develops early in life, the possibility of prenatal infection should be considered.

THE LEUKOCYTIC FORMULA IN THE TUBERCULOUS CONSIDERED AS A REACTION TO DAMAGED TISSUE. E. M. MEDLAR, Tubercle **9**:218, 1928.

The leukocytic reaction in any disease is regarded as a response to damaged tissue. A leukocytic picture is not regarded as pathognomonic of tuberculosis, and every type of leukocyte must be considered. There is no constant leukocytic formula in tuberculosis. The more nearly a patient maintains a septic leukocytic picture, the more grave is the prognosis. The marked and sustained increase of the neutrophil indicates formation of a tuberculous abscess. The more nearly a tuberculous person maintains a nonseptic leukocytic formula, with decrease of neutrophils, increase of lymphocytes and variation in the mononuclear leukocytes and eosinophils, the better the prognosis, as the tuberculous lesions are not progressing to abscess formation, but toward healing. The leukocytic picture established by one count a week over a period of months gives valuable information relative to the pathologic state of the tuberculous process as a whole.

H. J. CORPER.

THE EVOLUTION OF TUBERCULOUS INFECTION IN CHILDHOOD. OBSERVATIONS ON CHILDREN ADMITTED TO A DISPENSARY OVER A PERIOD OF TWELVE YEARS. CHARLES R. AUSTRIAN, Tubercle **9**:229, 1928.

A study of a group of persons observed at intervals through a period of from eight to twelve years demonstrates that though the maximum opportunity for tuberculous infection of the child is present in the homes of the actively tuberculous, extradomiciliary and extramural foci are also important sources of early contamination. Variations of response to the Pirquet test indicate that the tuberculous infection may vary in degree from time to time, even when it has been acquired in earliest childhood. Roentgenographic evidences of moderate enlargement of the mediastinum, of slight fibrosis of the lungs or of old localized infiltration of the base of the lung may remain unaltered through a period of years, even when they are present in young children who react positively to the Pirquet test. The number of young persons exposed to the risk of tuberculous infection who will develop clinical tuberculosis is relatively small, if contact with the source of infection is removed, and good conditions of hygiene and diet are established.

H. J. CORPER.

EXPERIMENTAL TUBERCULOUS MENINGITIS IN RABBITS. JOYCE R. SIMPSON and S. ROODHOUSE GLOYNE, Tubercle **9**:305, 1928.

Single intravenous and intraperitoneal injections of adult rabbits with bovine tubercle bacilli fail to produce cerebral or meningeal lesions. In two instances (one adult and one young rabbit), multiple massive intravenous injections sufficed to produce small cortical lesions, but no general meningitis. There is no evidence that the brain, meninges or cerebrospinal fluid exert any bactericidal effect on the tubercle bacillus, and it appears probable that the cerebrospinal fluid circulation is effectively shut off from infection by tubercle bacilli circulating in the blood stream.

H. J. CORPER.

PRIMARY ASPERGILLAR SPLENOMELAGY. P. FLANDRIN, P. EMIL-WEIL, PAUL CHEVALIER and R. GREGOIRE, Sang. **1**:509, 1927.

The present memoir on aspergillar splenomegaly by Emil-Weil and his associates is a detailed description of this condition, reported by him previously in the *Annales d'anatomie pathologique* and abstracted in the ARCHIVES OF PATHOLOGY (**5**:149, 1928). The article, which is almost a monograph, contains 100 pages and considers the minute clinical, pathologic and bacteriologic details of this type of splenomegaly. It also contains a full bibliography on this disease.

B. M. FRIED.

A STUDY OF TUBERCULOSIS IN INFANTS. M. A. ASSERSON, Tubercle **9**:323, 1928.

The incidence of tuberculous infection found among infants in attendance at hospitals was considerably higher than among those enrolled in clinics and health stations. It was also appreciably higher among infants who lived in the more crowded and congested sections of the city than among those from less congested districts. Apparently, in New York City, the incidence of infection among infants of various race and nationality stocks is similar, their ability or inability to resist infection, however, corresponding more nearly to that of the race and nationality to which they each belong. The infant's resistance to tuberculous infection is higher than it was formerly believed to be, but his resistance to tuberculous disease is relatively low, a fact long since recognized. Infants continuously exposed to tuberculosis in the home fall an easy prey to the disease. Segregation of the person with the disease or removal of the infant to another environment is necessary.

H. J. CORPER.

THE RESISTANCE OF THE CONJUNCTIVA TO THE PASSAGE OF PATHOGENIC AND NONPATHOGENIC ORGANISMS. MARIO MAGNASCO, Pathologica **20**:17, 1928.

The conjunctival mucosa defends itself from external agents by means of a combination of mechanical, chemical and biologic methods. The saprophytic germs, even when they are deposited on the conjunctival mucosa in exceptionally large quantities, cannot be cultivated in the aqueous humor. On the other hand, the virulent pathogenic organisms remaining in contact with the normal mucosa for an adequate period of time are able to gain entrance and finally enter the blood stream. However, such germs must be virulent in sufficient numbers and must find the right conditions, otherwise they behave as saprophytic organisms. The organisms have been recovered and cultivated from the aqueous humor of the eye and from the blood.

A. J. SALLE.

AMINO-ACID CONTENT OF THE BLOOD IN TUBERCULOSIS. L. HANTSCHMANN and M. STEUBE, Klin. Wchnschr. **7**:637, 1928.

Among 200 patients with pulmonary tuberculosis, an increase of the amino N of the blood is associated with new infections and exacerbations, and low values with cachexia or persistent emaciation.

AUTHORS' SUMMARY.

ANTHRACOSIS AND TUBERCULOSIS OF THE LUNGS. T. WEDEKIND, Klin. Wchnschr. **7**:890, 1928.

Tuberculosis, usually fatal in rabbits, was healed by the intravenous injection of an especially modified suspension of carbon dust. This is explained on the basis that stimulation of the endothelium by the carbon particles causes a marked migration of the phagocytes in the lungs. The so modified phagocytes are present in the lesions.

AUTHOR'S SUMMARY.

THE BIOSCOPIC REDUCTION TEST IN THE STUDY OF BACTERIAL LIFE PROCESSES. OTTO KIRCHNER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **52**:108, 1927.

Quantitative estimations of the catalase content of *Staphylococcus aureus*, *B. pyocyaneus* and *B. coli* grown in sugar, sugar-free, solid and liquid mediums show a great fluctuation of the catalase content. Acidity markedly lowers the catalase content as does anaerobiosis. After ten days' growth, there is a constant increase in catalase. In order to obtain bacteria with the lowest possible "rest-reduction," which is necessary for the bioscopic reduction test, a sugar-free fluid medium is necessary. The relation between peroxide formation and catalase content, on the one hand, and methylene blue reduction on the other, is discussed.

PAUL R. CANNON.

COMPARATIVE FILTRATION TESTS OF THE VIRUS OF CHICKEN PLAGUE AND OF TRANSMISSIBLE LYSIN. E. BERGER, Ztschr. f. Hyg. u. Infektionkrankh. **108**:315, 1928.

Berger has made careful comparative tests of the permeability of collodium membranes of varying concentration for the filtrable virus of chicken plague and a coli bacteriophage. He comes to the following conclusions: The units of the virus of chicken plague suspended in dilute serum are retained quantitatively by a 3 per cent collodium membrane. If one reduces the collodium concentration to 2.5 per cent, only a few virus particles pass the filter. When the collodium concentration decreases to 1.5 per cent one observes a sudden increase of the virus particles in the filtrate which amounts to at least 100 or even a 1,000 times. Only a 10 per cent collodium membrane retains the lysin particles completely. The concentrations which permit the passage of few lysin particles lie between 4.25 to 9 per cent. A sudden increase in the passage of lysin particles takes place below 4.25 per cent. The particles of the bacteriophage are evidently smaller than those of the chicken plague virus, but they are not as small as some investigators have maintained. It is, of course, impossible to determine whether the bacteriophage particles which are retained by the filters are the last autonomous units of the lysin. They may be absorbed by colloid particles. The possibility remains that the lysin itself consists of units no larger than molecules and therefore is an inanimate material.

W. OPHÜLS.

DEVELOPMENT OF PULMONARY ANTHRACOSIS IN CHILDHOOD. M. J. MADLENER, Ztschr. f. Tuberk. **49**:321, 1928.

This study was undertaken in order to determine whether certain parts of the lung show a predilection for the deposition of anthracotic material. Lungs of forty-four children between the ages of 2 months and 6 years were examined. The result of these studies was that there is no definite predilection in early anthracosis. In the majority of the lungs, deposition of coal pigment was found, even or nearly even, throughout both lungs. Deviations from this general condition were frequently due to pathologic lesions such as scars, adhesions and inflammatory changes. The frequency of such changes in the apical parts of the lungs in adults, explains the fact that in later years the apical parts show a predilection for anthracosis. But the primary seeding appears to occur at approximately equal density throughout the parenchyma.

MAX PINNER.

CONTRIBUTION TO THE PROPAGATION OF TUBERCULOSIS BY INFECTED MATERIALS. H. BRAEUNING and WANKEL, Ztschr. f. Tuberk. **50**:21, 1928.

Fourteen patients with open pulmonary tuberculosis were instructed to prepare for various types of needle work and to cough frequently on the material on which they worked, but never to expectorate on it. The material infected in such a way was soaked in saline solution, cut into small pieces and again soaked and squashed in the saline. The saline solution was then centrifugalized for one-half hour at high speed, and the sediment of each was injected into two guinea-pigs. None of these animals developed tuberculosis. These observations would indicate that droplets dried on various material are not infectious.

MAX PINNER.

CYTOLOGIC EXAMINATION OF FRESH SPUTUM. E. SALOMON, Ztschr. f. Tuberk. **50**:65, 1928.

It is important to pay more attention to cells found in sputum. Sputum containing alveolar epithelium cells must come from the parenchyma. If such sputum is found to be consistently free of tubercle bacilli, it is of more importance than in the absence of alveolar cells. The number of alveolar cells goes parallel with the amount of destruction. Serial examinations are diagnostically and prognostically important.

MAX PINNER.

TECHNIC OF CULTURES OF B C G. A. CALMETTE, Ztschr. f. Tuberk. **50**:124, 1928.

This is a detailed and technical discussion of the topic indicated in the title.

MAX PINNER.

Immunology

PREVENTION AND MODIFICATION OF MEASLES WITH ANTIMEASLES DIPLOCOCCUS GOAT SERUM. ARCHIBALD L. HOYNE and SILBER PEACOCK, Am. J. Dis. Child. **35**: 1021, 1928.

Observations on the use of Tunnicliff's immune goat serum showed it to have undoubted prophylactic value if administered within four days of exposure. If given at any time during the incubative period, it seems to exert a modifying influence on the disease, such as preventing complications and diminishing the death rate.

PEARL ZEEK.

A NOTE ON EXUDATIVE AND PRODUCTIVE PROCESSES IN PLEURAL TUBERCULOUS INFECTION. MAX PINNER, Am. Rev. Tuberc. **17**:627, 1928.

In rabbits, made allergic to tuberculin by previous injections of living or heat-killed tubercle bacilli, the response to intrapleural infection by living bovine bacilli is characterized by an exaggeration of the exudative reaction and by an acceleration of productive processes, as compared with normal rabbits. Neither the nonallergic nor the allergic tissue reaction is purely productive or purely exudative.

H. J. CORPER.

OCCURRENCE OF ALLERGY IN FIVE GENERATIONS OF ONE FAMILY. ABIGAIL ELIOT SMITH, Arch. Int. Med. **41**:472, 1928.

A statistical report is given of allergic manifestations occurring in five generations of a family and including ninety-four persons. The clinical presence of asthma, hay-fever, vasomotor rhinitis as evidenced by repeated spells of sneezing in the absence of infection, urticaria, angioneurotic edema or eczema, was taken as evidence of allergic tendency. Information was obtained by correspondence, with the use of a questionnaire. A discussion with bibliography is given of the factors

of heredity involved in the occurrence of hypersensitiveness. Smith interprets the hypersensitive tendency as a dominant factor.

In the family studied, 56.2 per cent of the members were allergic. Only one of twenty-three persons related to the family by marriage gave evidence of allergy. It is considered that allergy tends to occur in families with hereditary predisposition.

H. R. Fishback.

Relapse Phenomena of Spironema Recurrentis. Henry Edmund Meleney, J. Exper. Med. **48**:65, 1928.

Squirrels and chipmunks were found to be susceptible to infection with *Spironema recurrentis* of North China, but relapses did not occur in normal animals. Splenectomy caused an increase in the intensity of the infection and the appearance of one or two relapses. By inoculating splenectomized squirrels with a single human strain of spirochetes, six different strains were produced as a result of relapses. The strains all retained their specific agglutinating characteristics during the period of observation. The oldest strain was observed for 132 days and through thirty-eight transfers, the strain last isolated for twenty-two days and through six transfers. The sequence of strains in relapses was not always the same. Sometimes new strains were produced; at other times there was reversion to an older strain. Certain relationships between strains were established. The related strains were always those which appeared, or theoretically might have appeared, in alternate attacks. In only one case did related strains appear in two consecutive attacks. Squirrels whose spleens were not removed had only one attack and developed immune substances only against the strain inoculated or against closely related strains. It was possible by reinoculation to produce infection in such squirrels with as many as four different strains in succession. The spleen has a protective influence against the development of the relapse in the squirrel, but apparently does not control the formation of specific immune substances. An explanation of the clinical course of relapsing fever is suggested on the basis of the observations recorded. There is, at present, no justification for the division of the spirochetes of relapsing fever into difference species.

Author's Summary.

Factors Involved in the Production of Immunity with Pneumococcus Vaccine. Alvan L. Barach, J. Exper. Med. **48**:83, 1928.

The antigenic function of a pneumococcus vaccine made from the intact cell was compared with that derived from a watery extract of the cell free from formed elements. In each instance, the immunity produced was dependent on type-specific protective substance and not on the elaboration of the common protein antibody. The vaccine made from the intact cell resulted in both active and passive immunity which began on the third day, increased markedly to the fifth and remained approximately stationary to the seventh day. In the case of the Berkefeld filtrate of the shaken bacteria and the filtrate of the broth culture, the immunity began on the fourth day, increased to the fifth and remained approximately stationary to the seventh day. The immunity produced by pneumococcus type I vaccine is greater than that produced by type II. On the third day, mice vaccinated with type I vaccine resisted 100,000 minimal lethal doses, whereas mice immunized with type II resisted 10,000 minimal lethal doses. On the fifth day, a larger percentage of mice survived these doses than on the third day. Certain factors related to the preparation and dosage of the vaccine are discussed. As far as the time interval and the degree of immunity produced are concerned, these results suggest the possibility of employing pneumococcus vaccine in suitable doses in the treatment of lobar pneumonia. That an earlier activity of the immunity mechanism could actually be initiated in a patient with lobar pneumonia has still to be demonstrated.

Author's Summary.

RECIPROCAL EFFECTS OF CONCOMITANT INFECTIONS. LOUISE PEARCE, J. Exper. Med. **48**:125, 1928.

Experiments are reported in which it was shown, first, that the effectiveness of the reaction to experimental syphilis was increased in rabbits immune to vaccine virus, the ensuing disease being less severe than in control animals. It was further shown that a comparable modification of the syphilitic reaction occurred in rabbits inoculated intratesticularly with a mixture of *Treponema pallidum* and vaccine virus, and it was suggested that this result was due to the influence of a vaccinal immunity and to the local effect of the vaccinal reaction. It was pointed out that the manner in which two concomitant experimental infections are induced is of significance in determining the character of the host's reaction.

AUTHOR'S SUMMARY.

AGGLUTINATION BY PRECIPITIN. SECOND PAPER. F. S. JONES, J. Exper. Med. **48**:183, 1928.

The fact is shown that collodion particles sensitized with various proteins adsorb sufficient protein to agglutinate in the presence of precipitin specific for the adsorbed protein. The amount of adsorption, judged by agglutination, is not dependent on the concentration of the sensitizing protein beyond a certain maximum. The agglutination resulting from the addition of immune serum to particles sensitized with protein is immunologically specific. Particles exposed to a number of antigenic substances in succession are agglutinated by all of the appropriate antiserums. Particles exposed to immune serum and subsequently washed fail to agglutinate in the presence of antigen, although some of the protein constituents of the immune serum are fixed on them and its antibody content diminishes.

AUTHOR'S SUMMARY.

THE EFFECT OF INJECTIONS OF EPINEPHRINE ON THE HEMOLYSIN FORMATION IN NORMAL RATS. J. MARMORSTON-GOTTESMAN and DAVID PERLA, J. Exper. Med. **48**:225, 1928.

Large amounts of epnephrine injected repeatedly before and after the injection of antigen depress the antibody forming capacity of normal adult albino rats to a marked degree. The depression in antibody formation is roughly proportional to the quantity of epinephrine injected. Small amounts have no detectable effect on antibody formation.

AUTHORS' SUMMARY.

LOCAL SKIN REACTIVITY TO B. TYPHOSUS CULTURE FILTRATE. GREGORY SHWARTZMAN, J. Exper. Med. **48**:247, 1928.

A phenomenon of local skin reactivity to *B. typhosus* culture filtrates is described in this report. The reactivity was induced by skin injections of the filtrate followed twenty-four hours later by an intravenous injection of the same filtrate. The local response consisted of severe hemorrhagic necrosis and was fully developed from four to five hours after the second injection. The mechanism of the phenomenon described has not been fully studied as yet. An experimental comparison of it with the manifestations of bacterial allergy of the skin is necessary. There are certain features, however, which considered together distinguish this phenomenon from the known phenomena of bacterial hypersusceptibility. These features are: local reactivity; the short incubation period necessary to induce the local reactivity; the short duration of the state of reactivity; the ability to induce local reactivity by a single skin injection; the severity of the reaction, and the necessity to make the second injection of the toxic agent by the intravenous route.

RECIPROCAL AGGLUTINATION AND ABSORPTION OF AGGLUTININ TESTS WITH FIFTY-FOUR STRAINS OF HEMOLYTIC STREPTOCOCCI FROM PUERPERAL FEVER. FRANK L. MELENEY, HELEN ZAYTZEFF, HAROLD D. HARVEY and ZUNG-DAU ZAU, J. Exper. Med. **48**:299, 1928.

A study has been made by means of agglutination and absorption of agglutinin tests, of the antigenic relationships of fifty-four strains of hemolytic streptococci associated with an epidemic of puerperal fever in an obstetric hospital in a large city. These organisms were cultured from the blood, vaginas, peritoneal cavities and metastatic foci of patients, from the noses and throats of patients and staff members and from the peritoneum and axillary abscess of two of the nurses. In preliminary agglutination tests twenty-one of thirty-one cultures from the patients and nine of twenty-three cultures from the hospital personnel, fell into a single agglutinative group. By reciprocal agglutination and absorption of agglutinin tests, six of these strains were demonstrated to be antigenically identical. Two of these came from patient's blood cultures, three from patients' vaginal cultures and one from the nasal culture of one of the nurses. This nurse, however, was not the original carrier, for she did not join the hospital staff until after the epidemic began. By agglutination and complete absorption of agglutinin, sixteen more strains showed their antigenic similarity to, if not identity with, these other six strains. Four of these sixteen came from metastatic foci, and two from the vaginas of four of the five patients who had yielded from another source, the identical strains mentioned in four. Nine came from other patients and one from the axillary abscess of a nurse. No reciprocal tests were possible with these sixteen strains because serums had not been prepared against them. Five other strains which completely absorbed agglutinin had evidently lost their ability to agglutinate. Two of these strains were from the blood cultures and two from the vaginal cultures of two fatal cases. One was from the peritoneal cavity of a nurse. Two of these five strains were injected into rabbits in the usual way for the purpose of producing agglutinating antiserums, stimulated the production of serums which agglutinated completely the six antigenically identical strains but failed to agglutinate the homologous strains used to produce the respective serums. These two strains therefore retained the antigenic properties first of stimulating the agglutinating antibody in normal animals and second of uniting with the agglutinating antibody in the serum, but lost the ability to bring about the second phase of agglutination, namely, flocculation. We have demonstrated, therefore, a more or less permanent splitting of the phenomenon of agglutination.

AUTHORS' SUMMARY.

SEROLOGICAL DIFFERENTIATION OF STERIC ISOMERS. K. LANDSTEINER and J. VAN DER SCHEER, J. Exper. Med. **48**:315, 1928.

The experiments reported bring a definite proof for the view that the steric configuration of antigenic groups is one of the factors determining serologic specificity. In the particular case under consideration the mere difference in the position of H and COOH sufficed to alter the reactivity. The fact that steric isomers are acted on selectively by immune serums may be supposed to play a significant part in the serologic specificity of carbohydrates such as those discovered in bacterial antigens.

THE RELATION OF THE CAPSULAR SUBSTANCE OF B. COLI TO ANTIBODY PRODUCTION. THEOBALD SMITH, J. Exper. Med. **48**:351, 1928.

The relation between a strain of *B. coli* and its mutant with reference to the production of agglutinins and protective antibodies may be expressed by the statement that the original strain when injected into cows develops antibodies toward both itself and the mutant, whereas the mutant produces them only toward itself. The results point to the capsular substance as the material carrying virulence or, expressed somewhat differently, the factor which protects the micro-organism in the host.

AUTHOR'S SUMMARY.

VIRUS NEUTRALIZATION EXPERIMENTS WITH ROSENOW'S AND PETTIT'S ANTIPOLIOMYELITIC SERA. FRED W. STEWART and PETER HAZELBAUER, J. Exper. Med. **48**:449, 1928.

The Rosenow antistreptococic poliomyelitis serum concentrated or unconcentrated does not neutralize the virus of poliomyelitis as tested in monkeys. The Pettit antipoliomyelitis horse serum neutralizes the virus only occasionally. "Immune" sheep serums prepared according to the method of Pettit have not neutralized virus even when the normal serums of the same animal have given neutralization. The reason for such chance neutralizations is obscure and should not be confused with the constant virus-neutralizing action of both human and monkey convalescent serums. Experimental evidence affords no basis for the use of either the Rosenow or the Pettit serum in the therapy of poliomyelitis.

AUTHORS' SUMMARY.

VIRUS III, WITH SPECIAL REFERENCE TO THE RESPONSE OF IMMUNIZED RABBITS TO REINOCULATION. C. H. ANDREWES, J. Path. & Bact. **31**:461, 1928.

Virus III is far less common as a natural infection of laboratory rabbits amongst the stock used at Hampstead in 1927-1928 than in New York in 1923-1924. Rabbits with a solid immunity to virus III showed an almost constant but comparatively small local histologic reaction when the testes were reinoculated. Nuclear inclusions were absent from the lesions. Rabbits could be partly immunized, or sensitised, by giving a small dose of virus or an apparently neutral serum-virus mixture by various routes. Such rabbits, when tested three weeks later with active virus, showed more pronounced lesions in the interstitial tissues of the testis than did either normal or solidly immune rabbits. The lesions, however, were usually free from nuclear inclusions. This reaction is considered as a form of allergy. Virus III inoculated into the testes of solidly immune rabbits was not recovered two hours after inoculation. In partly immune rabbits the virus survived for forty-eight but not for seventy-two hours. Virus III inoculated into the testis of a guinea-pig was recovered after twenty-four but not after forty-eight hours. It survived for four days in mouse sarcomas and carcinomas, though continued propagation in these tumors was not found possible. The longer survival in a refractory species than in immune subjects of a susceptible species is considered as evidence that the immune animal in some way actively renders the virus noninfective. Similar observations recorded in other virus diseases are considered. Allergic reactions in virus infections are discussed in relation to the phenomena described in rabbits sensitized with virus III.

AUTHOR'S SUMMARY.

MULTIPLE ZONES IN SERUM PRECIPITATION. NEIL E. GOLDSWORTHY, J. Path. & Bact. **31**:525, 1928.

The method of optimal proportions in the precipitation reaction has been extended so that a wide range of ratios between antigen and antibody is secured. The occurrence of multiple zones of rapid particulation has been demonstrated with some antiserums. The position of the supernumerary zones within a series of mixtures of antigen and antiserum has generally been in those mixtures in which the amount of antigen was excessive relative to the amount in the optimal mixture.

AUTHOR'S SUMMARY.

THE PART PLAYED BY ALCOHOL IN THE WASSERMANN REACTION. C. H. BROWNING and E. M. DUNLOP, J. Path. & Bact. **31**:541, 1928.

In the Wassermann test when heated serums are used, differences in the sensitiveness, that is, complement-fixing power, of extract emulsions which vary in their alcohol content are usually related directly to the alcohol concentration. Increase in the concentration of alcohol in a Wassermann extract emulsion is, however, not associated with any regular and definite increase in its sensitiveness

when tested with any serum. Such an effect is only apparent in the presence of certain serums; and these constitute a small proportion of all serums examined. The results do not affect the observation that one serum may react more strongly positive with one lipoid extract, another with another. AUTHORS' SUMMARY.

TUBERCULOUS PLEURESIES FOLLOWING ARTIFICIAL PNEUMOTHORAX. E. RIST and T. VEBER, Ann. de méd. **24**:153, 1928.

Rist and Veber affirm that the pleural exudate which often follows compression of a tuberculous lung is basically the result of an allergic process. They find similarities between the inflammatory exudate present in the chest in patients with pneumothorax and the peritoneal fluid produced experimentally during intraperitoneal reinfections of tuberculous guinea-pigs. When a normal guinea-pig is infected intraperitoneally with a moderate dose of tubercle bacilli, the germ is rapidly phagocytized, and the insignificant peritoneal exudate is promptly absorbed. There is no intraperitoneal bacteriolysis. The animal, of course, develops ultimately a tuberculous peritonitis accompanied by a generalized tuberculosis. But when a tuberculous guinea-pig is superinfected with Koch's bacillus by way of the peritoneum, there occurs instantaneously a severe inflammatory reaction. In most instances, the animal succumbs within from eight to twenty-four hours with toxemic symptoms. The peritoneal exudate in these animals shows phagocytized tubercle bacilli which are disfigured and which have lost their acid-fastness. They, moreover, disappear with rapidity from the ascitic fluid. In instances in which the superinfected animals survive from this shock, their peritoneum shows no tuberculosis. Here, also, the animal perishes from his primary infection. It is interesting that the ascitic fluid from the superinfected animal often fails to cause tuberculosis in normal animals; and, in cases in which it does tend to phthisis, the disease runs a rather torpid course. This is obviously due to the destruction of the tubercle bacilli. (Allen K. Krause believes that the allergic inflammatory reaction interferes with the dissemination and possibly with the multiplication of the tubercle bacilli.) Rist and Veber by examining pleuritic fluids found that the bacilli present therein strikingly resemble those seen in the ascitic exudate already referred to. Here also they disappear as times goes on which is the result of their destruction. It is true that the pleural fluid produces tuberculosis in a guinea-pig. But the disease in the animal develops with an extreme slowness as compared with the richness of the fluid in "bacillary forms." The cultivation of tubercle bacilli from pleural fluid is successful in a small percentage of cases. And then if there are differences between some properties of the two fluids, it is possibly due to the fact that in the guinea-pigs the superinfected material is exogenous, whereas in man it is endogenous, coming from the patient's own lung. In the animal, the superinfection is single; in man it is probably continuous. It is possible then that in the latter the "equilibrium between the degree of allergy and the reinfecting dose is not properly adjusted." Basically, however, it is an allergic phenomenon. The article of Rist and Veber also deals with the occurrence of postpneumothoracic pleuresies with their cytology and with their evolution.

B. M. FRIED.

INVESTIGATIONS OF IMMUNE REACTIONS IN INVERTEBRATES (SIPUNCULUS NUDUS). J. CANTACUZENE, Arch. roum. de path. exper. **1**:1, 1928.

Corpuscles from sheep injected into the celom of *Sipunculus nudus*, a marine animal with a nonglandular thin walled alimentary tract suspended in a corpuscular celomic fluid, are resorbed by phagocytic ameboid cells—both the hyalin "amebocytes" (lymphocytes) and those with fine eosinophilic granules (amphophils); and the resorption is aided by "urns" which agglutinate the cells with their viscous secretion. The "vésicules énigmatiques," curious multinuclear cells which at first are unresponsive, later complement the action of the urns, and the amebocytes collected about them resemble endothelium. This agglutination creates a cytopenia promptly followed by an overproduction of all cells. After the first injection, the urns become more strongly agglutinative and acquire hemolytic properties for the

adherent sheep cells. The débris from the lysis is taken up by the phagocytes, which assume a giant cell formation, and in this viscous mass even the hematocytes of the sipunculus may be caught. At the same time, a hemolysin for the sheep cells appears in the plasma. This cellular agglutination is commented on as a possible coagulation phenomenon, for otherwise the fluid does not clot and there is no fibrin.

AUTHOR'S SUMMARY.

EXPERIMENTAL STUDIES OF ANTHRAX INFECTION AND ANTIANTHRAX IMMUNITY. D. COMBIESCO, Arch. roum. de path. exper. **1**:81, 1928.

The negative chemotaxis demonstrated for encapsulated anthrax bacteria and normal leukocytes depends on both the capsule and the bacterium; attenuated bacteria even when encapsulated are phagocytosed, as are nonencapsulated virulent bacteria, but there is no phagocytosis of virulent encapsulated bacteria. The delay in phagocytosis of bacteria in the skin may account for the greater susceptibility of this organ to infection. Only a short period is needed for the bacteria to "animalize" itself and so escape being engulfed. The lung, spleen, liver and blood are susceptible to infection though in a less degree than the skin.

AUTHOR'S SUMMARY.

CONTRIBUTIONS TO THE STUDY OF THE DISTRIBUTION OF BLOOD GROUPS IN ITALY. ETTORE CUBONI, Bull. dell' Instit. Sieroterapico Milanese **7**:153, 1928.

The author determined the blood group of 1,500 persons of whom seven tenths were natives of Northern Italy, one tenth of Central Italy and two tenths of Southern Italy, and attained the following percentage values: group O, 45.6; group A, 40.46; group B, 10.6, and group AB, 3.33. Biochemical index of race = 3.10.

A. J. SALLE.

THE IMMUNIZING ACTION OF "KOKTOIMMUNOGENS" OF TUBERCLE BACILLI. R. TORIKATA and K. IMANA KI, Beitr. z. Klin. d. Tuberk. **68**:306, 1928.

The "Koktoimmunogen" is prepared as follows: A culture of tubercle bacilli on glycerinated broth, having grown from three to four weeks, is heated in a boiling water bath for one-half hour. The boiled broth is then filtered through a candle. To the filtrate 0.5 per cent carbolic acid is added. The parenchymatous injection of this preparation into the one lung of guinea-pigs renders the injected lung considerably resistant to a later virulent infection. This immunization is much better tolerated than immunogen which is not boiled. The other organs show a more or less pronounced immunity against a tuberculous infection. The degree of immunity parallels the amount of immunogen injected. The immunogen is more easily absorbed and produces a higher state of immunity than the bacilli themselves, and they are less toxic. "Koktoimmunogens" are used preferably for immunization, not only in tuberculosis but in other infectious diseases as well.

MAX PINNER.

NONSPECIFIC TUBERCULIN REACTIONS FOLLOWING TREATMENT WITH OLD TUBERCULIN IN NONTUBERCULOUS PERSONS. J. HÄMEL, Beitr. z. Klin. d. Tuberk. **68**:345, 1928.

Twelve patients, free from tuberculosis, could be rendered sensitive to tuberculin by intensive subcutaneous treatment with old tuberculin. The maximal dose was 1,000 mg. The reactions obtained are not typical tuberculin reactions; they do not light up after renewed subcutaneous injection, and they are histologically different. They represent a protein hypersensitiveness.

MAX PINNER.

THE TESTICULAR TUBERCULIN REACTION IN GUINEA-PIGS. H. G. LEDERMANN, Beitr. z. Klin. d. Tuberk. **69**:265, 1928.

This reaction is done best with 0.1 cc. of a tuberculin solution of from 1:50 to 1:500. A positive reaction is manifested by enlargement and hardening of

the injected testicle. It was positive in all cases of definite tuberculosis when a sufficient amount of tuberculin was injected, and when the animals were not in the antiallergic or cachectic stage. The testicular reaction is followed in some cases by atrophy of the organ. The reaction was never positive in nontuberculous guinea-pigs. The specificity of the reaction is proved, too, by the fact that it does not occur after the injection of trichophytin and a nonspecific milk preparation. The testicle usually reacts sooner than the skin, rarely later. The testicular reaction does not show any type specificity.

MAX PINNER.

HYPERSENSITIVENESS AGAINST GLYCEROL BROTH. W. KELLER and W. DÖLTER, Beitr. z. Klin. d. Tuberk. **69**:444, 1928.

The concentrated glycerol broth, Höchst, as used as diagnostic control with old tuberculin, produces tuberculin-like reactions. The reasons for this activity could not be elucidated in spite of thorough studies.

MAX PINNER.

THE FERMENTATIVE METHOD OF PRODUCING DIPHTHERIA TOXIN IN VITRO. W. M. SDRAWOSMISSLOW and N. E. KOSTROMIN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **54**:1, 1927.

The authors believe that antibodies result from a molecular ungrouping of a combination of antigen and ferment. Trypsin mixed with diphtheria toxin in vitro resulted in an antiserum which had antitoxic properties. They consider that in infection the balance between the production of ferments and the antiferment action of the antigen determines the final result, all antibodies being merely changed ferments.

PAUL R. CANNON.

THE EXPERIMENTAL BASIS OF THE PROTECTION BY TOXOIDS. ST. BÄCHER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **54**:21, 1927.

This paper reviews the recent contributions to our knowledge of toxoids.

PAUL R. CANNON.

STUDIES OF HOG CHOLERA BACILLI. TOSHITERN HAYASHI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **54**:41, 1927.

Hayashi reports a study of sixty-two strains of so-called hog cholera bacilli as to agglutination types, variations and pathogenic significance.

PAUL R. CANNON.

FURTHER INVESTIGATIONS OF THE INHERITABILITY OF SUSCEPTIBILITY TO INFECTIOUS DISEASES. H. HIRSZFELDT and L. HIRSZFELDT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **54**:81, 1927.

Schick tests in 116 families and Dick tests in 55 families showed that when both parents gave positive reactions, most of the children gave positive reactions, whereas when both parents gave negative reactions, approximately two thirds of the children reacted negatively and one third positively. When one parent gave a negative reaction and the other a positive one, about one half of the children gave a positive reaction. The authors then discuss the theoretical aspects of these observations.

PAUL R. CANNON.

THE INFLUENCE OF CERTAIN AMINO-ACIDS ON CULTURES OF DIPHTHERIA BACILLI. B. SBARSKY and Z. JERMOLJEWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **54**:105, 1927.

In a previous paper, Sbarsky and Subkowa (*Biochem. Ztschr.* 161, 1925) showed that amino-acids, particularly tyrosine, inactivated diphtheria toxin. The

present authors report the effect on diphtheria bacilli. Small amounts of amino-acids in cultures do not kill the bacteria themselves, but tyrosine diminishes the toxicity of mildly toxicogenic cultures of diphtheria bacilli when injected subcutaneously. The effect is minimal, however, with toxic strains. Alanine, leucine and glycocol have no such effect. Tyrosine in 0.5 per cent concentration in broth cultures diminishes the production of toxin from ten to twenty times.

PAUL R. CANNON.

THE RELATION OF LIPOID ANTIBODIES IN THE FRACTIONATION OF SERUM BY ACID PRECIPITATION. G. E. SELTER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **54**:113, 1927.

Antiserum obtained by immunizing with lecithin or organ extracts and hog serum was fractionated with dilute hydrochloric acid. The hog serum antibodies were found in the albumin fraction, and the lecithin antibodies in the globulin fraction. Heterogenous lipoid antibodies were found in the albumin fraction. The author then discusses theoretical aspects of these results.

PAUL R. CANNON.

A STUDY OF ANTIBODIES IN THEIR RELATION TO GLOBULINS IN THE CEREBROSPINAL FLUID. ALFRED FLAUM, Acta path. et microbiol. Scandinav. **5**:16, 1928.

On local immunization of rabbits, hemolysins and agglutinins developed in the cerebrospinal fluid. As Pandy's reaction frequently was absent in fluid containing antibodies, it seems that antibodies may exist independently of globulins. By heating it was not possible to demonstrate any differences between the agglutinins in serum and in cerebrospinal fluid.

THE THOMSEN HEMAGGLUTINATION PHENOMENON. V. FRIEDENREICH, Acta path. et microbiol. Scandinav. **5**:59, 1928.

A brief review is given of earlier reports which show that the transforming agent, described by Thomsen, which acts on human corpuscles of every group so that they become agglutinable in all human serums, including AB serum and serum of the same iso-agglutinative group, is the filtrable product of a bacterium. This product without the bacteria does not cause "spontaneous" agglutination, and seems to be a catalysin agent not combined in the end-product. The specific receptor (called receptor T) and the correspond-agglutinin appearing under the influence of this substance apparently are independent of the isoreceptors A and B and agglutinins a and b, and the agglutinin is seemingly not identical with the cold-agglutinin as at first claimed by Thomsen. The "M" and "J" organisms isolated from blood belong to the coryneform bacteria. Other bacteria, a spirilla closely related to the cholera vibrion, and two luminous organisms had the same transforming power.

AUTHOR'S SUMMARY.

PROBLEMS IN PRODUCTION OF ANTIDIPHTHERIA AND ANTITETANUS SERUMS. S. SCHMIDT, Acta path. et microbiol. Scandinav. **5**:129, 1928.

The results of Ramon are confirmed.

THE CONSTANCY OF THE TITER OF HUMAN ISO-AGGLUTININS IN HEALTH AND DISEASE. KARL LEHMANN, Acta path. et microbiol. Scandinav. **5**:155, 1928.

In an apparently normal person there was no variation in the titer of the iso-agglutinin during one year. In thirty-four patients with chronic medical diseases no variations in the iso-agglutinin titer were observed; this was also the case in ninety-five patients with conditions which required surgical intervention; only in three patients with phlegmon of the lower extremity, suppurative enteritis and puerperal fever, respectively, was there a slight increase in the titer.

Tumors

TISSUE CULTURE OF INTRACRANIAL TUMORS WITH A NOTE ON THE MENINGIOMAS. FREDERICK E. KREDEL, Am. J. Path. **4**:337, 1928.

Successful tissue cultures with the hanging-drop technic have been made from meningioma, metastatic carcinoma and spongioblastoma multiforme. Phagocytic cells migrated out in large numbers in meningioma cultures. The ability of these cells to ingest particulate carmine decreased progressively with the age of the cultures.

AUTHOR'S SUMMARY.

TWO OSTEOBLASTOMAS NOT CONNECTED WITH BONE, HISTOLOGICALLY IDENTICAL WITH OSTEOGENIC SARCOMA, AND CLINICALLY BENIGN. C. P. RHOADS and HERMAN BLUMGART, Am. J. Path. **4**:363, 1928.

Two tumors of soft tissues not attached to bone are described which had a histologic structure identical with osteogenic sarcoma. These tumors have shown no recurrence after local removal. The cases lend support to the theory that the fibroblast in any part of the body may give rise to tumor bone.

AUTHORS' SUMMARY.

A DERMOID OF THE CORNEA IN A GUINEA-PIG. A. BRUNSCHWIG, Am. J. Path. **4**:371, 1928.

A case of true dermoid of the cornea in a guinea-pig is recorded. All dermoids reported as corneal in origin cannot be finally classified as such because of lack of microscopic evidence and because many of them were at the limbus with more of the tumor extending over sclera than cornea.

AUTHOR'S SUMMARY.

FIBROSARCOMA OF THE PLEURA. H. E. MACMAHON and G. K. MALLORY, Am. J. Path. **4**:387, 1928.

A case is reported of a primary fibrosarcoma of the pleura in an elderly male. The literature is briefly reviewed. The case is discussed as an unusual pathologic condition which warrants clinical consideration.

AUTHORS' SUMMARY.

MULTIPLE PRIMARY NEOPLASMS IN LOWER ANIMALS. WILLIAM H. FELDMAN, Am. J. Path. **4**:497, 1928.

From a brief review of the literature it is apparent that primary, multiple neoplasms may appear in a great variety of species. Multiple neoplasms are frequently noted in necropsies on dogs more than 10 years of age. This suggests the influence of senile retrogression and subsequent tissue involution in the etiology of these tumors. The possible influence of heredity is a factor which warrants investigation. A case of multiple primary neoplasms in a dog, aged 13 years, is presented. The tumors described are: recurrent squamous cell carcinoma of the mouth with metastasis to the regional lymph node; multiple hemangiomas of the liver; malignant leiomyoma of the cecum; papillary cystadenoma of the prostate, and a testicular tumor arising from the interstitial or Leydig cells. Multiple lymphphomatous nodules of the spleen are also present.

AUTHOR'S SUMMARY.

HYDROGEN ION CONCENTRATION OF BUFFER SOLUTIONS IN WHICH THE CHICKEN-TUMOR VIRUS REMAINS ACTIVE. MARGARET REED LEWIS and LEONOR MICHAELIS, Bull. Johns Hopkins Hosp. **43**:92, 1928.

The chicken-tumor virus remained active in buffer solutions having a hydrogen ion cencentration of from 4 to 12, but became inactive in buffer solutions of p_H 3 and in sodium hydroxide p_H 12.5.

AUTHORS' SUMMARY.

THE MAIN CHARACTERISTICS OF CARCINOMA CELLS IN VITRO. ALBERT FISCHER, Bull. Johns Hopkins Hosp. **43**:105, 1928.

Carcinoma cells are able to proliferate indefinitely among the dormant cells in the organism — because of certain humoral factors. The cells are able to build up unlimited masses of protoplasm from the serum constituent; and because of certain cellular factors, the cells are able to utilize the otherwise cell-type-specific growth principles, the desmones, from any cells in the organism. This fact explains the heterotopic growth of cancer cells in general. The rate of growth of tumor cell in vitro is markedly lower than that of the normal tissue cells under conditions which are optimal for the latter. Carcinoma cells are able to build up their cytoplasm from embryonic tissue juice which has been inactivated by subjection to a temperature of 56 C. for one hour. This indicates that carcinoma cells are able to utilize certain substances in a form different from that required by normal cells. Normal tissue cells are unable to overgrow carcinoma cells in vitro. Carcinoma cells are more sensitive to an increased hydrogen ion concentration than are normal tissue cells. Carcinoma cells are more sensitive to high and low tensions of oxygen than are normal tissue cells. The growth of carcinoma cells ceases at a low oxygen tension, whereas, under similar conditions, normal tissue cells continue to proliferate.

AUTHOR'S SUMMARY.

THE MALIGNANT FIBROBLAST OF JENSEN SARCOMA. ALEXIS CARREL and ALBERT H. EBELING, J. Exper. Med. **48**:285, 1928.

A pure strain of fibroblasts has been isolated from the Jensen rat sarcoma. The cells give rise to tumors on transplantation into animals and during several months of life in vitro have maintained their malignancy unchanged. The malignant cells are generally coarser and more refringent than normal cells. They possess the cytologic characteristics of fibroblasts without showing any morphologic abnormality. They can be considered as healthy cells. The texture of their colonies is looser than that of normal fibroblasts and sarcoma no. 10 fibroblasts. Their residual activity does not differ markedly from the normal. They proliferate unlimitedly in a nutrient medium. They liquefy the fibrin of rat plasma and turn phenol red golden yellow. They do not liquefy the fibrin of chicken plasma. They multiply in chick embryo juice, calf liver digest and also in rat serum. Their growth activity is increased by the presence of bone marrow. The unlimited growth of Jensen sarcoma within the body may possibly be attributed to the ability of the fibroblasts to maintain themselves on the substances present in rat serum. This property itself probably depends on the increased enzyme activity of the cells.

AUTHORS' SUMMARY.

THE EFFECT OF OXIDATION OF FILTRATES OF A CHICKEN SARCOMA. J. HOWARD MUELLER, J. Exper. Med. **48**:343, 1928.

The rapid autoinactivation at 37 C. of candle filtrates of the Rous Foul Sarcoma I is due in large part to oxidation. Such oxidation may be prevented by a dilution of 1:2000 cysteine hydrochloride brought to p_H with sodium hydroxide. The variability of infectiousness of candle filtrates is due in part at least to oxidative changes which take place during their preparation; and more active extracts may be obtained by preventing these by means of cysteine. For elaborate experiments it is possible to preserve filtrates in the icebox with cysteine and petrolatum until test inoculations have established their infectivity.

AUTHOR'S SUMMARY.

THE EFFECT OF RADIATION ON ROUS CHICKEN TUMOR. J. C. MOTTRAM, Brit. J. Exper. Path. **9**:147, 1928.

Experiments are described which show that radiation in vivo may make the tissues nonreceptive to the growth of a Rous tumor, or may cause an existing tumor

to disappear, but does not destroy the power of the cell-free filtrate from such a tumor to infect another bird. The explanation given is that the cell-free filtrate inoculated into scarified areas acts on the new, healthy fibroblasts to produce cancerous changes. If the fibroblasts are destroyed by radiation no cancer growth will be produced by inoculation. The author discusses also the variations among birds in the receptivity of other parts of the body to tumor growth after one tumor has been made to disappear by radiation. It seems that the serum of some of the birds develops the ability to destroy in vitro the power of cell-free filtrates to produce a tumor. This property is absent in normal serum.

PEARL M. ZEEK.

THE ACID-BASE EQUILIBRIUM AND THE IONIC EQUILIBRIUM IN CANCER AND IN THE BODY. A. P. DUSTIN, Arch. internat. de méd. expér. **3**:597, 1927.

A three-fold theory is presented which covers all the known aspects of spontaneous and experimental cancer. The three elements are (1) the cancer-producing factor, a virus or biologic agent, (2) the soil, and (3) the prevailing local lesion, or portal of entry. Although the etiologic agent still rests in obscurity, it is thought to be related to bacteriophages and lytic principles. The fact that proliferation apparently precedes and prepares the way for lysis, and that the cancer advances by means of karyokinesis and karyolysis fits in well with this theory. Twort has been struck by this analogy, and Carrel has noted the similarities between the cancer-producing agent and the hereditary lytic principles of Bordet.

PEARL M. ZEEK.

THE ACID-BASE EQUILIBRIUM AND THE IONIC EQUILIBRIUM IN CANCER AND PRECANCEROUS CONDITIONS. RENÉ READING, Arch. internat. de méd. expér. **3**:613, 1927.

The cancerous condition is characterized, in addition to the tumor formation, by marked humoral disturbances: alteration in the mechanism of the acid-base equilibrium, in the ionic equilibrium and in the sugar regulatory mechanism. These alterations are independent of the presence of a tumor, and they precede the appearance of the malignant disease. The origin of these alterations is principally familial. In cancer families there is "cancerable" soil in a proportion conforming with that of modern statistics of heredity. The excitement of humoral disturbances accelerates the development of the disease. Age exercises no influence on the alteration of acid-base or ionic equilibrium. The disturbance of the acid-base equilibrium in cancer is caused primarily by the break-down of the neutralization mechanism. The x-rays exercise their influence not only by their selective action on cells in mitosis, but also by their action on the biologic regulatory mechanism of cellular division: the reaction of the blood, the ionic equilibrium and sugar regulation. The parathyroid appears to be one of the factors in the regulation of the blood reaction. The modifications in these alterations, after one treatment by radiation, has a decided prognostic value. The simultaneous existence of plasma alkalinity, ionic disturbance and a hyperglycemic type curve may contribute usefully to the early diagnosis of cancer. The biologic laws which govern cell division in general, seem to hold equally well for division of malignant cells. In the second place, a certain degree of alkalinity and ionic disturbance appear to be necessary for the initiation and the continuation of the phenomenon.

AUTHOR'S SUMMARY.

GLYCOGEN, GLUCOSE AND LACTIC ACID CONTENT OF BENIGN AND MALIGNANT TUMORS. F. BERNHARD, Klin. Wchnschr. **7**:1184, 1928.

The sugar concentration of malignant and benign tumors is the same. Malignant tumors contain ten times as much glycogen as the benign. Mammary gland carcinomas always contain glycogen. The lactic acid content of malignant tumors is greater than that of benign. Variations in the concentration of lactic acid

in the blood has no diagnostic value in carcinoma. Whether the determination of the glycogen content of malignant tumors has any prognostic value for the surgeon, has yet to be determined.

AUTHOR'S SUMMARY.

TAR CANCER IN MAN. L. LAZZARINI, Gazz. d. osp. **49**:485, 1928.

Tar cancer as an industrial hazard was first reported in the Italian literature in 1926. Lazzarini reports a case occurring in a man, aged 67, who had been employed for many years in the distillation of tar. The patient was often burned on the forearms and hands by particles of boiling tar. In October, 1926, he developed a swelling on the back of his hand, which he attributed to a lesion produced by a rusty nail. On its becoming ulcerous, the patient applied zinc oxide ointment, but without avail. The ulceration was elliptical in shape, the larger diameter being about 8 cm. The margins were raised above the surrounding skin, and the appearance was that of a crater. The base of the ulceration had a fatty appearance and, when rubbed with gauze, did not bleed. The mass was movable and involved only the skin. The clinical diagnosis was: precancerous dermatosis due to tar. May 21, 1927, the tumor was removed under local anesthesia. Recovery was normal and, during the six months since the operation, no complications have arisen. The diagnosis based on the histologic observations was spinocellular epithelioma.

INFLUENCE OF FAT DEFICIENT DIET ON THE GROWTH OF TRANSPLANTED TUMORS. WARO NAKAHARA and EIICHI SOMEKAWA, Japan M. World **8**:125, 1928.

The almost total absence of fats and lipoids in the diet does not perceptibly affect the growth in albino rats of transplanted tumors, both sarcoma and carcinoma.

AUTHORS' SUMMARY.

Medicolegal Pathology

POISONING FROM SHOE DYE. L. SHINABERY, J. Indiana M. A. **20**:296, 1927.

Since the report of Muehlberger (*J. A. M. A.* **84**:1987 [June 27] 1925) of forty-seven cases of poisoning from shoe dyes collected from the literature to which he added nine, about six other cases have been reported. Most of these have been observed in this country, but the earliest report was from France, where about one fourth of all cases of poisoning from shoe dye have occurred. It is not always decided how much of the poisoning is due to aniline, and how much to nitrobenzine, since the dyes contain both, and the symptoms due to each are similar. Recovery followed the poisoning reported by Shinabery. The patient dyed his shoes one evening, wore them for a time, and also wore them the next morning. In the afternoon he had a peculiar color (methemoglobinemia), but no other symptom at that time. The subsequent course of events was characteristic for such poisonings when recovery is the outcome.

E. R. LE COUNT.

TWO CASES OF ACUTE YELLOW ATROPHY OF THE LIVER FOLLOWING ADMINISTRATION OF ATOPHAN. L. LOEWENTHAL, W. MACKAY and E. LOWE, Brit. M. J. **1**:592, 1928.

As a plea for caution in the administration of the drug variously known as atophan, phenylcinchoninic acid, cinchophen, quinophan, agotan or phenoquin, the authors warn against its uncontrolled use over a long period in any patient, in persons who exhibit an especial susceptibility and in those in whom there is a previous "hepatic derangement." They agree with G. Graham that the safest therapy with this drug is attained by four days of administration followed by four days of rest, but one of their deaths occurred in a patient who adhered rigidly to this regulation.

The first death they report was of a woman, aged 55, a sufferer from chronic rheumatoid arthritis, who on a neighbor's advice took three 7.5 grain (0.4924 Gm.) tablets of cinchophen daily from the middle of July, 1927, until Jan. 20, 1928. Illness then began with slight jaundice which gradually deepened and was followed in a week by delirium, coma and death. At the autopsy, the liver was typically yellow-ocher, with small reddish areas; it weighed 19 ounces (497.6 Gm.) and resembled a fluid jelly contained in a thin capsule. Microscopically, there was a moderate degree of multilobular cirrhosis which probably antedated the acute yellow atrophy. The great majority of liver cells were completely necrotic; some places showed fatty degeneration, but there was practically no attempt at regeneration. The second death was also in a woman, aged 55, who in the middle of December, 1927, began taking 7.5 grains of cinchophen, three times a day, three days a week on the advice of her physician, and continued on this routine until the end of January, 1928. On February 5, slight jaundice was noted, and this gradually deepened. On March 4, an exploratory laparotomy confirmed the cirrhotic condition of the liver, and death occurred the next day. The macroscopic and microscopic changes coincided with those of the first report barring a preceding mulitobular cirrhosis. The liver weighed 26 ounces (808.6 Gm.).

GEORGE RUKSTINAT

STATUS LYMPHATICUS AND SUDDEN DEATH. J. ANDERSON and J. A. M. CAMERON, Glasgow M. J. **108**:129, 1927.

This report is based on 100 cases in which a study of the gross disease was made in all the bodies, and of that histologically demonstrable in fifty. It is not stated over how long a period the observations extended. This is of considerable interest since thirty of the bodies were of children under 18 months of age who died when anesthetized for less than ten minutes with chloroform. The weight of the thymus, from 8 to 10 Gm., regarded as normal in Glasgow for the age of 2 years, is probably less than has been found normal in other places. Some disease was frequently present in the thyroid gland and in the suprarenal glands and the chromaffin cells of the medulla were fewer than normal.

Importance is attached to these changes, but still more to disseminated fatty degeneration in the intima of arteries of all sizes. Illustrating the significance of this disease, mention is made of death of two adults, aged 21 and 23 years respectively, from intracerebral hemorrhage and status lymphaticus with widespread fatty intimal arterial degeneration. In a third person, aged 16 years, some of the arteries involved served the nodes and muscles of the His bundle system in the heart, and there was paroxysmal tachycardia, with hemorrhages in the myocardium in addition to the systemic hyperplasia of the lymphoid tissue. The article is a valuable addition to the literature of this subject because of new evidence offered for the recognition of status lymphaticus and new suggestions to explain its mortality. Allusion is made to the possibility of a deficiency of epinephrine and the hypoglycemia which presumably would follow.

E. R. LE COUNT.

FATAL POISONING WITH ACETYLSALICYLIC ACID. L. DE JANKOVICH, Ann. de méd. lég. **8**:7, 1928.

Acetylsalicylic acid is certainly not thought of as a poison by any considerable portion of the large number of persons now habituated to its use in all civilized nations. Physicians know that doses of from 10 to 15 grains (0.65 to 0.972 Gm.) may have undesirable effects in aged persons or patients with heart disease. That it has attained some vogue as a means for committing suicide is not surprising, since it is accessible and not expensive. Janovich, who writes from the Medicolegal Institute of the University of Szeged, about 118 miles southeast of Budapest, refers to many poisonings in Hungary. He reports five deaths with amounts of from 20 to 75 Gm. (roughly from ¾ to 2½ apothecary ounces). Minute hemorrhages are found in the pleura, pericardium and endocardium, and the lining of the stomach

and bowel. The tissues of the body are acid; also the blood, cerebrospinal and other fluids. The large amount of salicylic acid released in the stomach acts as a mild caustic.

E. R. Le Count.

Asphyxia and Epinephrine. H. Asada, Ann. de méd. lég. **8**:22, 1928.

Experiments with rabbits and dogs demonstrated that the subpleural hemorrhages, "spots of Tardieu," caused by compressing the trachea do not have any relationship to convulsions. By opening one side of the thorax, their advent was seen before convulsions occurred. In animals in which the suprarenal glands have been removed, they were rarely observed as a result of asphyxia, but if epinephrine was injected into such animals before asphyxia, the hemorrhages formed.

These and many other experiments by Yosida at the Institute of Legal Medicine in Nagasaki, are reported by Asada, the Director. The lungs were removed from some animals and perfused with Ringer's solution, and then small amounts of epinephrine were added to the solution. Epinephrine was also injected into animals kept alive for a while after the lungs were removed. It was concluded that the systemic arteries and capillaries act differently to epinephrine than similar pulmonary vessels.

With vagisection, the hemorrhages were more abundant. This intimate relation to effect resulting from impulses through nerves of both the vagus and sympathetic systems, established by Asada and his assistants, was supported by Piédeliévre who, in discussion, mentioned the subpleural hemorrhages produced in guinea-pigs by eserine which first stimulates the sympathetic and then the vagus nerves.

E. R. Le Count.

Viability of the Newly Born. D. Macaggi, Ann. de méd lég. **8**:32, 1928.

In both civil and criminal legal procedures, the persistence of life after birth for even a few moments may be important. Questions of inheritance, survivorship and infanticide are intimately related to the manifestations of postnatal life and proofs that the child actually lived after birth. There are discriminations from both legal and purely biologic aspects, and there is a large literature in both law and medicine regarding these matters.

Observations are reported of strong and regular heart action continuing for two and one-half minutes after birth without respirations. From such apnea, asphyxia is said to differ by having weak and irregular heart beats. That problems of live or still birth are far from being simple is also illustrated by reports from some of the most competent pathologists of cases in which the lungs were devoid of air in the bodies of infants known to have cried out and lived as long as thirty hours after birth.

For a long time studies of these and closely related problems have been prosecuted at the Institute of Legal Medicine of the Royal University in Genoa under the director, Perrando, and particularly of changes of the glands of internal secretion which may cause death soon after birth. In this report by Macaggi, at the twelfth Congress of Legal Medicine at Lyon in 1927, he adds several others to the description he made at a previous annual meeting, of airless lungs from newly born infants. He insists that the infants never breathed although they lived several hours, and regards the condition of the lungs as arrested development.

Many who have discussed this prolongation of life without apparent respiration have maintained that some exchange of gases actually does occur within the lungs. An opposing group to which Macaggi belongs attach great importance to the relatively large amount of hemoglobin possessed by new-born infants, the number of erythrocytes, the differences of respiratory exchange in the tissue and the many intrinsic conditions which favor persistence of life at the birth epoch. They also suggest that there may be a compensatory exchange of gases with oxygenation of hemoglobin in the stomach, bowel and skin.

Supporting such views are the evidences of maldevelopment of the lungs found in some of these infants; for example, lungs which have attained a development corresponding to only the fourth month of intra-uterine life found in the body of an infant living some hours after birth at the eighth month of gestation. Of course, Macaggi refers to careful histologic studies and to the care used in demonstrating the presence or absence of air in these lungs, to the importance of stains for elastic tissue, the Bielchowsky stain for reticulum and the amount and distribution of the elements of the pulmonary framework.

When attempts are made to promote breathing by artificial respiration, the infundibula or terminal intralobular bronchioles marking the utmost passages for air in such lungs are found ballooned out a little, with many hemorrhages caused by trying to get air into lungs which do not possess alveoli. The pulmonary lobules may be masses of tubules and diverticula lined with cylindrical epithelium and buried in an abundant stroma. Where there should be air sacs, there are masses of reticulum. That infanticide, or attempted infanticide, may be concerned with a child born alive and with lungs subsequently found airless by most careful examination, and still later by microscopic study found devoid of development proportionate to that of the rest of the body, is a matter of grave significance.

E. R. Le Count.

Bismuth Poisoning. J. Gaté and H. Gardére, Lyon méd. **140**:109, 1927.

Intolerance developing during the course of treatment with compounds containing mercury, arsenic or bismuth is not uncommon. A large liver in patients addicted to alcohol seems to aid. Severe attacks of asthmatic dyspnea, many in each day with an unbearable generalized pruritis with erythema, ulcerative stomatitis and painful lassitude, followed the second dose of a series of injections of iodobismuthate of quinine for late manifestations of syphilis. An interval of one month had been allowed following a first series of a dozen injections. Recovery was protracted, and the cutaneous trouble was not altogether cured at the end of four months. Others have observed a similar intolerance for bismuth, but usually without such variegated symptoms. The authors urge an interval of two or three months between treatments.

E. R. Le Count.

Traumatic Hemorrhage Deep in the Brain. F. Reuter, Deutsche. Ztschr. f. Chir. **207**:92, 1927.

In seven brains hemorrhages from blunt force applied to the head were found in the basal ganglions, the pons or in the region of the internal capsule. Six of the brains were those of persons in advanced years; the seventh was that of a person so young that the cranial bones were still somewhat elastic and unbroken.

It is suggested that sclerosis of the blood vessels and high blood pressure may be factors contributing to such central traumatic hemorrhages. They are of unusual interest on account of their great rarity, the possibility of their confusion with delayed traumatic apoplexy and also because such central hemorrhages due to external violence and relatively unknown at present may be the starting point for the considerable hemorrhages which have already won recognition as delayed or late traumatic apoplexy.

It is also suggested that as much as six months should be allowed to lapse before deciding that injuries of the head have not included deep injuries of the brain which may prove to be serious. Symptoms of such central lacerations may be present immediately after disappearance of the symptoms of concussion or, with the secondary subsequent bleeding, they may develop weeks or months later. The disposition of the deep injuries, their location in line with the external injuries of the head and contusions of the outside of the brain, conform to the direction of the force applied to the outside of the head.

E. R. Le Count.

CHANGES IN THE BLOOD WITH CARBON MONOXIDE, A COMPOUND SOLUTION OF CRESOL AND ANILINE OIL POISONING. ARNETH and ALBACHT, Zentralbl. f. Gewerbehyg. **14**:225, 1927.

One of each variety of poisoning is reported. The patient poisoned with aniline recovered. Cellular and hemoglobin estimations of the blood were carefully made. With aniline, the red cells went down to 3,520,000 two days after the poisoning and leukocytosis was absent. The latter was present in the other two poisonings. There was also a marked diminution of red cells with the poisoning from a compound solution of cresol.

E. R. LE COUNT.

CARBON MONOXIDE POISONINGS IN GARAGES. MÄTJE, Zentralbl. r. Gewerbehyg. **14**:275, 1927.

During the past winter, a number of carbon monoxide poisonings in garages, with some deaths, have occurred in Hanover. Mätje, who is an engineer, calls attention to their prevalence in the cold months and in small garages built for a single motor vehicle. Some reports have been made by others on the amount of carbon monoxide in the exhaust gas from carefully controlled experiments. Those here reported have as their chief interest the great increase of carbon monoxide in the gas when there is no load on the engine. With the machine standing still, the carbon monoxide poured forth in exhaust gas is perhaps six or seven times greater than when running on a road with a load.

E. R. LE COUNT.

SERUM AND HEMOGLOBIN PRECIPITINS IN MEDICOLEGAL EXAMINATIONS OF BLOOD. K. FUJIWARA, Ztschr. f. d. ges. gerichtl. Med. **11**:253, 1928.

Precipitin serum for hemoglobin may be obtained without much more difficulty than for serum. The hemoglobin serum is the more precisely specific. In actual practice it is best to use both these precipitin serums at the same time because in extracting blood spots the hemoglobin may dissolve better than the serum at one time or vice versa.

LIPOIDS IN THE LIVER AT BIRTH. S. NUNOSE, Jap. Med. World **8**:8,1928.

In the stillborn, the amount of neutral fat is larger than in the infants born alive; it is also more evenly distributed in the cells of the lobules, and is found hardly at all in the Kupffer cells. By Ciaccio's method, positive reaction was obtained oftener in the stillborn than in those born alive. It is suggested that these differences in the distribution of lipoidal bodies may have medicolegal value.

Technical

STAINING FIBRILLARY NEUROGLIA IN FORMALIN-FIXED MATERIAL. LEO M. DAVIDOFF, Am. J. Path. **4**:493, 1928.

By the method described for which original article must be consulted it is possible to obtain excellent preparations to show neuroglia fibrils in material which, owing to its long fixation in formalin, does not yield itself to the satisfactory demonstration of these elements by any other means. The importance of this is evident when it is recalled that for numerous decades formalin fixation, especially for the central nervous system, has been not only the favorite, but, in many instances, the sole preservative employed. In dealing particularly with the gliomatous tumors of the nervous system where a reliable and specific method for the demonstration of glia fibrils is often essential for their classification, this method may serve to save much valuable material which might otherwise be wholly useless, or at least unsuited for thorough investigation in the light of recent developments in our knowledge of these neoplasms.

A Rapid Method for Staining Myelin Sheaths. Arthur Weil, Arch. Neurol. & Psychiat. **20**:392, 1928.

By the following method satisfactory staining can be secured within one hour of sections fixed in formaldehyde and cut frozen or embedded in celloidin or paraffin. The celloidin need not be removed. The paraffin should be removed before staining. Frozen sections should be placed in 70 per cent alcohol for from five to ten minutes and then put back into water. The sections should be from 20 to 30 microns in thickness. The procedure follows: From water bring sections into 5 per cent solution of potassium dichromate; then wash twice in tap water. Stain the sections for fifteen minutes at from 45 to 50 C. in mixtures of equal parts of fresh solutions of 4 per cent iron alum in water and 1 per cent solution of hematoxylin prepared from 10 per cent solution in absolute alcohol at least six months old. Wash sections in tap water again. Place sections in 4 per cent solution of iron alum in water until the gray matter or degenerated areas can be recognized from a white background. Then wash the sections three times in tap water and place them in the following solution: Borax 10 Gm.; potassium ferricyanide, 12.5 Gm.; distilled water 1,000 cc.; when sufficiently differentiated wash three times in tap water and dehydrate in 95 per cent alcohol and then in absolute alcohol-ether and xylene and mount in balsam.

A New Procedure for Determining Blood Sedimentation Rates. E. D. Plass and M. D. Rourke, J. Clin. Investigation **5**:531, 1928.

A new method is detailed for the study of red blood cell sedimentation rates. Dry heparin is used as anticoagulant. Results are expressed as the percentage of total possible settling which occurs on standing one hour. Total possible settling is the plasma volume percentage determined by centrifugalizing twenty minutes.

H. R. Fishback.

A Method of Inoculating Blood Plates for the Identification of Hemolytic Streptococci. Eugenia Valentine, J. Infect. Dis. **43**:167, 1928.

A method is described of inoculating blood plates by cutting the agar with the infected loop, and then moving the loop under the agar in a small section of the plate prior to the surface streaking. After eighteen hours' growth a hemolytic zone is produced where the organism has grown under the agar, although colonies on the surface of the plate may not be definitely hemolytic even after forty-eight hours' incubation. The greater ability of streptococci to hemolyze red blood cells when inoculated under the agar or when grown in blood agar pour plates is ascribed, in part, to the partial anaerobiosis.

Four strains of beta hemolytic streptococcus are described which, when first cultured from the human source, failed to show hemolysis on surface-streaked plates but appeared greenish by transmitted light.

Author's Summary.

A New Culture Medium for Bacillus Tuberculosis (Human). G. Vercellana, Pathologica. **20**:129, 1928.

A new medium, for the isolation and cultivation of human tubercle bacilli, is described. The medium is composed of cow's milk, emulsion of whole eggs, neutral double distilled glycerin, and basic fuchsin. This preparation was found superior to Petroff's in ease of preparation, cheapness and results obtained. Preliminary steps in the treatment of sputum and the concentration of the organisms are the same as those followed by Petroff.

A. J. Salle.

Copper Peroxidase Reaction of Leukocytes. H. Sakurada, K. Shoji and K. Suzuki, Tohoku J. Exper. Med. **11**:1, 6 and 10, 1928.

Sakurada's paper describes an application of the copper peroxidase reaction of Sato and Sekiya to the differentiation of eosinophil and pseudo-eosinophil cells.

Both in smears and by the counting chamber method, the true eosinophil cells are stained dark violet, while the pseudo-eosinophils appear red and other leukocytes are unstained.

Using the simple oxidase reaction first described by himself *(Tohoku J. Exper. Med.* **10**:293, 299 and 305, 1928; ARCH. PATH. **6**:756 [Oct.] 1928), Shoji differentiates similarly in smears and by the counting chamber method the eosinophil and pseudo-eosinophil cells. The granules of the true eosinophils are stained intensely violet, while the pseudo-eosinophils appear dark red with or without a few granules stained faintly violet.

In fresh blood smears the myeloid leukocytes are negative to the peroxidase reaction, while they remain positive to the oxidase stain (striatal blood picture). When they are kept carefully, the oxidase reagent may be kept as long as three weeks, while the peroxidase reagent retains its potency as long as half a year.

EDWIN P. JORDAN.

TESTING THE FUNCTION OF THE LIVER BY MEANS OF CONGO-RED. Y. NINOMIYA, Tohoku J. Exper. Med. **11**:151 and 188, 1928.

Of known dyes congo-red is best suited for testing the function of the liver. On intravenous injection congo-red is eliminated from the blood more slowly in patients with diseases of the liver and in animals, the liver of which has been injured experimentally, than in normal persons and animals. The determination of the rate of elimination of congo-red after intravenous injection may be regarded as of clinical value. The rate of elimination of congo-red appears to be independent of the reticulo-endothelial apparatus because the elimination is delayed only when the function of the parenchymatous cells of the liver is diminished.

Book Reviews

DENTAL INFECTION AND SYSTEMIC DISEASE. By DR. RUSSELL L. HADEN, with a foreword by DR. E. C. ROSENOW. Pp. 165. Philadelphia: Lea & Febiger, 1928.

This small volume presents in connected form the research work bearing on the relation of dental infection to systemic disease, done during the past six years, at the Deaner Institute in Kansas City, Mo., and the Department of Experimental Medicine of the University of Kansas School of Medicine. It is therefore the report of much experimental work and prolonged clinical observation of a large number of patients suffering from a variety of diseases. Dental focal infection is the keynote, and the experimental evidence is arranged in support of the theory of "elective localization."

That "the fundamental conceptions of focal infection have been thoroughly accepted by clinicians everywhere" is, I believe, more or less true, but as to the principles involved there are differences of opinion. The concept of focal infection is not affected if experimental intravenous inoculations in animals fail to reproduce lesions in the same tissue as in the patient from whom the streptococcus was isolated. If bacteria from infected foci are sufficiently virulent to localize in any tissue of an animal, even under the highly artificial conditions of intravenous inoculation, this fact in itself would support the significance of the process of focal infection. The particular tissue involved in the patient or the animal depends on many factors of stress and strain in individuals and groups. In this book, however, the theory of elective localization is so intimately incorporated with each phase of the subject that it tends to tinge the results reported and, therefore, somewhat detracts from the value of many important observations.

In table 12, page 90, the results of 1,750 injections in animals are shown. The lesions occurred in various tissues in the following order: joint, kidney, muscle, stomach and duodenum, endocardium, eye, myocardium, brain. The evidence for elective localization (the number of animals showing lesions in a particular tissue being taken as a basis) is that the streptococci in 8 per cent of the lesions of the kidneys were from patients with infection of these organs; 9 per cent of the endocardial lesions, from those with heart or vascular disease; in 43 per cent of the lesions of the eyes, from patients with infected eyes, and in 25 per cent of the lesions of the stomach and duodenum, from those with peptic ulcer. This gives an entirely different aspect to the results as tabulated in percentages by the author.

The chapter on the incidence and types of dental infection associated with sysemic disease emphasizes the importance of infection about pulpless teeth, with or without evidence in dental films, and further points out that mere removal of the infected teeth will effect little if the accompanying alveolar condition is not also treated. The incidence of root fragments and of foreign bodies, noted after extraction, is found to be particularly important. The pathologic observations in the various types of focal infection, and the factors favoring systemic infection from these infected foci, are treated inadequately. A special chapter should have been devoted to these subjects to make the book more valuable as a reference work.

The chapter on the bacteriology is well written. It shows clearly the importance of streptococci. Under the head of the types of systemic disease associated with chronic dental infection, the author discusses the significance of true secondary infection, the effect of absorbed toxins and the development of allergic states. Chapter VI, fully illustrated, covers the results of intravenous injections in rabbits. Chapter VII, which takes up about 40 per cent of the whole volume, is devoted to rather detailed clinical histories, and to the results of intravenous injection of streptococci from typical cases, illustrating the relation of dental infection to systemic disease. Here, however, the credulity of the reader is often strained to the breaking point. For example, it is reported that a culture from an area

of residual infection in the bone of the jaw caused an onychia in the right toe of the left forefoot of an inoculated rabbit. This is illustrated, and just below, in figure 60, is shown an involved thumb on the left hand of the patient.

The book contains valuable material, but the reader must discount certain of the conclusions because of Dr. Haden's firm conviction that the theory of elective localization explains the phenomena.

FILTERABLE VIRUSES. By HAROLD L. AMOSS, JACQUES J. BRONFENBRENNER, ALEXIS CARREL, EDMUND V. COWDRY, RUDOLF W. GLASER, ERNEST W. GOODPASTURE, LOUIS O. KUNKEL, STUART MUDD, PETER K. OLITSKY and THOMAS M. RIVERS. Edited by THOMAS M. RIVERS. Cloth. Price, $7.50. Pp. 428, with 27 illustrations. Baltimore: Williams & Wilkins Company, 1928.

The term "filterable viruses" is used by the authors merely as the preferred one of a number of unsatisfactory names designating "certain active transmissible agents which are capable of producing pathologic conditions in bacteria, plants, insects, fish, birds and mammals." The diseases which have been attributed to such etiologic agents are listed. Topics of fundamental importance, such as general aspects of filtrable viruses, filters and filtration, tissue cultures in relation to the viruses and intracellular changes in virus diseases, occupy several chapters. Easily variable factors, such as the size of the filter pores, the electrical charge and the technic of filtration, together with the possibility of filtrable bacterial forms (pleomorphism, dissociation), complicate the classification of such active agents on the basis of filtrability alone. Another important possibility is that these unseen agents may not be living, even though they require living tissue for the demonstration of their presence, but they may be chemical or enzymatic, affecting cellular mutations. The so-called pathologic states are then the evidence of the newly acquired functions. Clinical recovery from a virus disease is usually followed by a lasting immunity, which may be due, it is asserted, to prolonged infection. This is in contrast to the general behavior of bacterial diseases. The difficulty of generalizing for a group of diseases so little understood is obvious. Each of the remaining chapters reviews one virus disease for mammals, fowls, insects, bacteria, etc., respectively. The discussion of poliomyelitis, the example chosen for man, includes the history, clinical picture, pathologic anatomy, etiology (with little credence given to the claims for a causative streptococcus, and a conclusion that the relationship of the "globoid bodies" to poliomyelitis remains unsolved), natural and acquired immunity, epidemiology and treatment. The great clinical and economic importance of these diseases makes them of special concern to both practicing physicians and investigators, who will find the articles and the extensive bibliographies of this book valuable.

ELEMENTARY BACTERIOLOGY. By JOSEPH E. GREAVES, M.S., PH.D., Professor of Bacteriology, Utah Agricultural College and ETHELYN O. GREAVES, M.S. Price, $3.50. Pp. 506, with 129 illustrations, including several colored plates. Philadelphia: W. B. Saunders Company, 1928.

A conscientious attempt has been made here to present bacteriology in a virtually narrative style. It begins in a cafeteria on an autumn day; later on, one finds that water is, next to life, the most marvelous thing in the world; on the last page, all the children of the community march down the street, while blindfolded men fire into the crowd. By such means, and by the introduction of historical matter, an interest is built up, almost elaborately, in each topic presented.

The authors attempt a survey of the whole field by touching only the outstanding matter under the various heads. It is futile, therefore, to expand or to qualify to any great extent. As a result, any teacher of bacteriology will feel that many really important topics are so briefly presented as to lead to misconceptions. Antitoxins and vaccines, for example, are covered in eleven pages. The chapter on immunity barely skims the surface. The chapter on milk includes twenty pages, of which the consumption of milk occupies a page and the value of milk two and one-

half pages, whereas pasteurization covers less than a page, and the organism of tuberculosis is not mentioned in this connection. But space is repeatedly allowed for such remarks as that there are roughly 3,000,000,000,000 malarial parasites in the body during a severe attack, and that this is fifty times greater than the number of seconds which have elapsed since the birth of Christ.

Although fact has frequently given up its place to fancy, the act has thus been deliberate. The authors have chosen strenuously to impress the fact rather than to explain it. They have not intended that the work go further than to give the reader an elementary knowledge. It has little value as a reference book, but it might well be placed on the shelves of a general library. Teachers of bacteriology as incidental to other major subjects will find use for the volume. Too much ground is covered superficially, perhaps, for its use as a textbook in elementary courses preceding further work in bacteriology.

KONSTITUTIONSSEROLOGIE UND BLUTGRUPPENFORSCHUNG. By LUDWIG HIRSZFELD. Paper. Price, 18 marks. Pp. 235, with 12 illustrations. Berlin: Julius Springer, 1928.

The author recognizes a growing interest in blood grouping and properly warns against the present popularization in which simple correlation with disease and adaptability to purely statistical studies is looked for by physicians and anthropologists. In his mind, the blood group relations involve the most complex fundamental principles of immunity, and the organism as a whole may be regarded as an instrument of endless capabilities for serologic adjustment; he uses the word constitutional in the sense of depending on the hypothetic genotype. Individually specific serologic properties exist, he holds, which are predetermined and heritable, and the immunologic responses of the organism by no means merely reflect the infinite variety of outside stimuli but move in a predetermined direction. Normal antibodies he considers to be predetermined biochemical organs whose phylogenetic origin and ontogenetic development correspond with those of anatomic characteristics. "Serogenesis" is, then, analogous to morphogenesis; the formation of immune bodies is the unfolding and growth of the cell capabilities as governed by the genotype.

Following an outline of the principal facts concerning antibodies, are chapters on the heritability of group specific substances touching both theoretical and experimental work; the phylogenetic origin of the properties responsible for iso-agglutination and their anthropologic significance, the physiologic and immunbiologic bases of iso-agglutination and auto-agglutination, the subgroups, specific group differentiation during ontogenesis, the chemical characteristics of the iso-agglutinable substances of human blood, their relation to pathology and the theoretical foundations of constitutional serology. The bibliography is excellent, and, like many of the discussions, if not complete in every respect, it profits by intelligent selection.

FORENSISCHE GYNÄKOLOGIE VON PROF. DR. FRITZ REUTER. Sonderabdruck aus Biologie und Pathologie des Weibes. Vol. 8, part 40. Berlin: Urban & Schwarzenberg, 1928.

This book presents forensic gynecology in its various aspects and with specific reference to criminal and civil laws and regulations in Germany and Austria. Not only are the medicolegal relations and duties of the physician as gynecologist and obstetrician considered in detail, but also the criminal psychology and criminality of women, marriage rights including such matters as impotence and questions of paternity, and then, of course, punishable acts of all sorts against morals and modesty, including rape, sexual perversions, abortions in various phases and infanticide. The various anatomic and other examinations that must be made in the effort to solve particular problems of the nature just indicated are described clearly with reports of illustrative cases. The statement that the performance of

the lung test in the case of the new-born is subject to definite official regulations in all countries should be modified by adding "except in the United States." The statement on page 1201 that the electric current is said to be used especially in America for producing abortion is hardly in accord with the actual facts. The book is recommended as a valuable guide in its field.

THE NEWER KNOWLEDGE OF BACTERIOLOGY AND IMMUNOLOGY. By Eighty-Two Contributors. Edited by EDWIN O. JORDAN and I. S. FALK. Cloth. Price, $10. Pp. 1196. Chicago: The University of Chicago Press, 1928.

Such is the accumulation of detailed studies within the period of only a few years and such the multitude of suggestions for further work, that the greatest need appears to be for discernment of essentials and guidance to a profitable choice of problems. Without selection and direction, the mass of material will become overwhelming. This book, furnishing "the landmarks of progress" and suggestions of unsolved problems, will assuredly be welcomed by all workers in the field. The book devotes forty-eight chapters to bacteria, covering their morphology, their chemical structure, and their growth; special consideration is given to bacterial associations and dissociation, to oxidation and reduction of bacteria, their metabolism, their enzymes, and to their relations to food, soil fertility, water supply, sanitation and diseases. These chapters handily represent a stupendous investigative effort. Thirty-five chapters encompass the newer knowledge of immunology—a research of no less apparent immensity. Each chapter is, further, a revelation of what must be dealt with as further work appears. The typing and arrangement are clear and simple.

MANUEL DE BACTÉRIOLOGIE MÉDICALE. PAR ANDRE PHILIBERT, Professor agrégé de bactériologie, Chef des travaux pratiques de bactériologie à la Faculté de médecine de Paris. 1 volume. Price, unbound 45 francs. Pp. 552, with 21 colored illustrations in the text (120 figures). Paris: Masson & Cie, 1928.

The object of this work is to present an easy and accurate reference book on the common bacteriologic problems arising in the practice of medicine. This purpose is fulfilled admirably. It is written in a clear and lucid way but so organized that it does not give the best kind of introduction to basic bacteriologic principles.

The first portion is devoted to a brief discussion of the technic of the study of bacteria and includes an especially good chapter on the p_H of the culture mediums. It is followed by good description of the methods used in collecting material and examination bacteriologically of the sputum, feces, blood, urine and pus with emphasis placed on the organism most common in each. The illustrative plates are extremely good. The last two thirds of the book is devoted to the identification and significance of the principle, known, causative organisms of disease of the human being. There is throughout a praiseworthy simplification of methods.

MIKROMETHODEN ZUR BLUTUNTERSUCHUNG. IVAR BANG. BEARBEITET VON GUNNAR BLIX. Sixth edition. Pp. 54, with 8 figures. Munich: J. F. Bergmann, 1927.

The sixth edition of Bang's book has been ably brought up to date by Blix. Many methods are changed and new technical hints are added, while the booklet is shortened. This abbreviation was possible because the methods now do not need evidence as to exactness, as they did when Bang began working. In the clinical routine work, the methods elaborated by Folin are simpler. However, if only small amounts (drops) of blood are available, as in the case of small animals, or when a large series of examinations is made at short intervals, Bang's methods stand supreme.

Books Received

FORENSIC MEDICINE. A Text-Book for Students and Practitioners. By Sydney Smith, M.D., D.P.H., Regius Professor of Forensic Medicine, University of Edinburgh. With an introduction by Prof. Harvey Littlejohn, F.R.C.S., F.R.S.E. Second edition. Cloth. Price, $8. Pp. 602, with 166 illustrations. Philadelphia: P. Blakiston's Son & Company, 1928.

The first edition of this valuable book was reviewed with commendation in the ARCHIVES (2:299, 1926). In the meantime, the author has become the regius professor of forensic medicine in the University of Edinburgh, succeeding the late Harvey Littlejohn who wrote the introduction to the book. The revision is thorough. The chapter on fire-arms and projectiles has been enlarged notably. About fifty new illustrations have been added. While especially adapted to the legal procedure in England and Scotland, Smith's "Forensic Medicine" will be found of great value and interest to students of forensic medicine everywhere.

BULLETIN OF THE NATIONAL RESEARCH COUNCIL: No. 64. The Coroner and The Medical Examiner. Issued under the Auspices of the Committee on Medicolegal Problems. By Oscar T. Schultz and E. M. Morgan, with a supplement on Medical Testimony by E. M. Morgan. Price, $1.50. Pp. 101. Washington, D. C.: The National Research Council of The National Academy of Sciences, 1928.

FOSSIL LIZARDS OF NORTH AMERICA. By Charles W. Gilmore. Volume XXII, Third Memoir. Pp. 201. Washington, D. C.: National Academy of Sciences, 1928.

DIE BEDEUTUNG DES RETIKULOENDOTHELIALSYSTEMS FÜR DAS STREPTOKOKKENSEPSISPROBLEM. By N. Louros und H. E. Scheyer. Price, 14 marks. Pp. 101, with 17 illustrations. Leipzig: Georg Thieme, 1928.

In this monograph are summarized the results of extensive experiments on streptococcus infection in mice, with particular reference to the rôle of the reticuloendothelial system. It was found that by the introduction of certain nonspecific substances the resistance of mice to streptococcus infection could be increased, and this effect is ascribed to the action of the substances in question on the reticuloendothelial system. Unfortunately, some of the substances used bear proprietary names. It is proposed to apply the same principles to the study of streptococcus infections in man.

PREVENTIVE MEDICINE. By Mark F. Boyd, M.D., C.P.H., Member of Regular Field Staff, International Health Division of Rockefeller Foundation; formerly professor of bacteriology and preventive medicine in the Medical Department of the University of Texas. Third edition, revised. Cloth. Price, $4.50. Pp. 475, with 151 illustrations. Philadelphia: W. B. Saunders Company, 1928.

This new edition of a well known text and reference book on preventive medicine has been thoroughly revised and is well up to the previous standards. It can be used equally well by the beginning student as a text and by the more advanced for reference. The chapters on microbic and parasitic infections are particularly good. This book can be highly recommended for use in medical schools and in the allied fields.

RECENT ADVANCES IN CHEMISTRY IN RELATION TO MEDICAL PRACTICE. By W. McKim Marriott, B.S., M.D., Dean and Professor of Pediatrics, Washington University School of Medicine; Physician-in-Chief, St. Louis Children's Hospital. Lectures of the San Diego Academy of Medicine, Series of 1927. Cloth. Price, $2.25. Pp. 141, with 12 illustrations. St. Louis: C. V. Mosby Company, 1928.

The subjects of the lectures are: fundamental chemical considerations, acidosis and alkalosis, chemistry of the blood, foods and metabolism (two lectures) and the endocrines. The lectures discuss clearly and stimulatingly fundamental chemical conceptions on which are based recent applications of chemistry to clinical pathology.

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